PANCREATIC DIGESTION

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STUDIES IN

PANCREATIC DIGESTION

MADE WITH PANCREATIC JUICE.

BY

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PREFACE.

This book is a reprint of a series of research articles originally printed in various medical journals.

These studies were made with rabbits' pancreatic juice, and the conclusions here presented differ somewhat from those arrived at by other investigators, working with pancreatic extracts.

Since these differences of opinion exist, I have thought best to reproduce these papers in a convenient form, that they may be more accessible to students of intestinal digestion.

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I.—INFLUENCE OF BILE ON THE FAT-SPLITTING PROPERTIES OF PANCREATIC JUICE.

In the spring and summer of last year, in the Berlin Physiological Laboratory, I made a study of the fat-splitting properties of pancreatic juice and read a paper on this subject before the physiological section of the Tenth International Medical Congress. The complete and more detailed presentation of this work is the object of this paper.

The short paper on emulsion, apart from any interest or value that may attach to this portion of the paper itself, is of importance because of its bearing on the methods used in the study of pancreatic juice.

EMULSIONS.

In 1870 E. v. Brucke[1] announced the fact that when rancid oil[2] is shaken with a solution of sodium carbonate and certain other alkaline fluids an immediate emulsion results. He believed that the oil was broken into fine globules by the shaking and that the soap formed served to hold the emulsion by preventing the union of the oil globules.

In 1878 Johannes Gad[3] called attention to the fact that when oil containing the proper percentage of fatty acid was placed on the surface of a carbonate of sodium solution a beautiful spontaneous emulsion resulted, and from this he held that neither shaking nor any other outside mechanical force was necessary to the formation of an emulsion, but that the chemical force developed by the soap formation was of itself sufficient under favorable circumstances to break the oil drops into the finest emulsion globules. There is but little room for doubt, I think, that Gad is right in this opinion. In fact, the only question which might

1 *Sitzungsbericht der Wiener Acad. der Wissensch.*, Bd. lxi, II. Abth., p. 362.

2 By rancid oil is meant oil containing fatty acid.

3 *Archiv f. Anat. u. Physiol.*, 1878, p. 181.

arise is whether the force developed by the soap formation is not a physical (Quincke) rather than a chemical one. Gad also believed with Brucke that the soap formed had much to do with holding the emulsion, and this proposition is, I think, now everywhere accepted, although opinions differ widely as to the manner in which the soap acts in bringing about this result.

I wish here to call attention to the method used by Gad in his study of spontaneous emulsion, since this method is the basis of the methods used by me in the study of the fat-splitting properties of pancreatic juice.

A $\frac{1}{4}$ per cent. carbonate of sodium solution is placed in a series of watch-glasses, and drops of oil containing different percentages of fatty acid are gently placed, by means of a pipette, on the surface of the fluid in the watch-glasses. The amount of spontaneous emulsion in the various glasses is carefully noted and compared, and in this way one can readily ascertain the percentage of fatty acid required to give the best emulsion.

It must, of course, be remembered in this connection, that the percentage of fatty acid required to give the maximum amount of spontaneous emulsion will vary with other conditions, such as temperature, strength of soda solution, etc., and that therefore only experiments made under similar conditions can be compared. By this method Gad observed that under otherwise similar conditions a certain definite percentage of fatty acid must be present in oil to give the maximum amount of spontaneous emulsion. For example, he found that with a $\frac{1}{4}$ per cent. carbonate of sodium solution at room temperature, about $5\frac{1}{2}$ per cent. of fatty acid was required, and that with increasing or diminishing per cents. of acid above or below $5\frac{1}{2}$ per cent. he got less and less emulsion, until finally there was no emulsion at all. A very little more or less than $5\frac{1}{2}$ per cent. of acid gave an incomplete emulsion. He found, therefore, that the limits of good spontaneous emulsibility were not only constant but also quite narrow, and upon these important facts depends the value of his method.

We have in Gad's method a simple and accurate means of determining the proper percentage of fatty acids for giving the best spontaneous emulsion of any given oil under given conditions.

After repeating the experiments of Gad and confirming his observations I devoted considerable time to the study of the influ-

ence of shaking and other outside mechanical means on the formation of emulsions.

The oil used almost exclusively in my experiments was olive oil that had been neutralized by shaking for two hours with a saturated solution of barium hydrate at a temperature of 95° C. and then pipetted and filtered. Oil freshly prepared in this manner will be found practically neutral, and the term neutral olive oil as used in this paper always refers to such oil.

The stirring was done chiefly by currents of air carried from a blowing machine into the liquids to be stirred by means of rubber tubing and glass rods. This method is not only more convenient, but it has other advantages over the ordinary one of shaking the tube.

My experiments led me to the following conclusions:

1. No amount of stirring will give a permanent emulsion of either neutral olive oil or of rancid olive oil in distilled water. (Frey[1] found differently.)

2. No amount of stirring will give a permanent emulsion with neutral olive oil and a ¼ per cent. carbonate of sodium solution.

3. Shaking rancid oil and a ¼ per cent. carbonate of sodium solution gives a good permanent emulsion, even though the oil contain a very small or a very large percentage of fatty acid.

From the above observations we see that when the conditions for soap formation are present, shaking very much widens the range of good emulsibility and promotes the formation of a good permanent emulsion, but when the conditions for soap formation are not present, the shaking has no influence whatever.

In our study of emulsions we must remember that two things are necessary to the formation of a good permanent emulsion:

1. The oil must be broken into very fine globules.

2. These globules must not only be prevented from running together, but they must also remain rather uniformly distributed throughout the liquid. Now since we know that soap and certain other materials, as albumen and mucilage, have the power of holding emulsions, it would seem an easy matter to make a mechanical emulsion by shaking neutral oil in a solution of soap, albumen or mucilage; but such in truth is not the case. In my experiments with soap solution and neutral olive oil I found that in very heavy solutions of soap, by violent and prolonged stirring,

1 *Archiv f. Anat. u. Physiol.*, 1881, p. 382.

I could get only an imperfect emulsion, one in which the oil globules were larger and more variable in size than those formed by spontaneous emulsion.

These mechanical emulsions do not approach in perfection a physiological emulsion, such as milk; and they can be formed only in very viscous liquids and with such great mechanical force as to place them beyond the pale of physiological importance.

For the study, therefore, of the influence of stirring in the formation of good permanent emulsions, such as may have some physiological importance, we must return to the experiments already noted, where a moderate amount of stirring very much hastened and promoted the formation of good emulsions when the conditions for soap formation were present.

The influence of stirring under such circumstances may, I think, be explained as follows. When too little acid is present for the formation of a good spontaneous emulsion, the shaking or stirring simply favors the emulsion by promoting soap formation. It breaks the oil into a number of small globules which are constantly presenting new surfaces to the surrounding alkaline fluid, thus enabling the soda to combine with all the fatty acid present, in the formation of soap, and the chemical force thus liberated by the soap formation becomes an important factor in the breaking of the oil drops into the fine emulsion globules, just as it does in pure spontaneous emulsion.

When too much acid is present for good spontaneous emulsion, the process is brought to a stand-still by the formation of a heavy soap membrane between the oil drop and the alkaline fluid, thus preventing further soap formation. Under these conditions, shaking breaks the oil drop and consequently the soap membrane, thus constantly presenting new surfaces of oil to the surrounding alkaline fluid and in that way favoring soap formation and the resulting emulsification. We see, therefore, that while shaking may play a very important rôle in the formation of emulsions, its action is chiefly an indirect one, promoting emulsification by favoring soap formation, and that the chemical force liberated by this process is the force most active in breaking the oil drops into fine emulsion globules. From my experiments I formulate the following general law concerning the influence of stirring in the formation of emulsions.

The amount of stirring required to give a good emulsion of

oil in a ¼ per cent. carbonate of sodium solution will be in inverse proportion to the nearness with which the percentage of fatty acid in the oil approaches the proper percentage for giving the maximum amount of spontaneous emulsion. If the oil contains the exact percentage of fatty acid for giving the best spontaneous emulsion, then the shaking will be superfluous, since a good emulsion will form without motion and no amount of shaking can improve it. If, on the other hand, the oil be entirely free from fatty acid, then, as we have seen, no amount of shaking will give a good emulsion. Between these two extremes the above law applies, and shaking may contribute very largely to the formation of emulsions.

In the application of the above principles we have a simple and convenient method of determining when an oil is practically free from fatty acid; viz., shake it with a ¼ per cent. solution of carbonate of sodium, and if there be no fatty acid present, the mixture rapidly clears.

By the same method we may tell when we have fatty acid free from admixture with oil; viz., shake the fatty acid with the soda solution, and if oil be present we will have more or less milky whiteness, which is characteristic of emulsions; but if no oil be present, we will have a simple cloudiness due to the insoluble soap formed. From all that has been said, it follows as a logical conclusion that the energy required to make an oil emulsible will be in direct proportion to the stability of the oil molecule of the given oil. The more stable the oil molecule, the more energy required to split it into fatty acid and glycerine. It matters not whether the energy be in the form of heat or of organized ferments, bacteria, or of unorganized ferments, as the fat-splitting ferment of the pancreas.

During my experiments I found that heating neutral olive oil developed fatty acid and made it emulsible, and that if this heated oil be again neutralized it became non-emulsible, thus showing the emulsibility to be due to the acidity. I also found that the greater the heat and the longer applied, the more fatty acid was developed, so that boiled olive oil contained too much acid for good spontaneous emulsibility.

It is an interesting fact that the acids freed by heating various oils seemed to have greater power in making them emulsible than a like quantity of oleic acid. This is especially true of castor

oil. Castor oil is not made more emulsible by the addition of oleic acid, but after boiling it may be emulsified by shaking it with sodium solution, but it never becomes spontaneously emulsible; this latter fact Gad called attention to and thought it due to the viscosity of this oil. The stability of the castor oil molecule is shown by the great heat required to develop sufficient fatty acid to give an emulsion. These facts seem to indicate that the fatty acids of an oil are the fatty acids best adapted for giving emulsibility to this particular oil.

It is a physiological fact beyond dispute that the splitting of fats is a most important preliminary step in fat digestion. That the cooking of fats will develop in them fatty acid is therefore a fact of considerable physiological importance, and one that, so far as I know, has not previously been noticed.

As I have previously intimated, it is my belief that the chemical force developed by soap formation is the chief factor in the formation of all physiological emulsions, that it plays quite as important a rôle in the formation of the emulsion as the soap does in holding it after it is formed.

That soap has the property of holding emulsions is, I think, an undisputed fact, but the manner in which the soap acts is a question concerning which there has been much difference of opinion. In explanation of this difficult problem I wish modestly to express my belief in a theory of emulsions which is a modification of that offered by Gad. Gad believed that the fine globules of oil were coated as soon as formed with insoluble soap particles which formed a protecting envelope that prevented the oil drops from running together. The modification which I offer is as follows: the chemical process of soap formation which breaks the oil into fine globules must develop considerable heat; this must necessarily have the effect of bringing a certain amount of otherwise insoluble soap into solution. This heat will necessarily be local and felt chiefly just at the point where the soap is formed, and all the surrounding liquid will be cooler. The soap, therefore, which is brought into solution by the heat either is precipitated a moment later on coming in contact with cooler parts of the liquid, or it causes increased viscosity in the liquid. We may therefore say that the heat is developed, the soap formed and dissolved and the oil broken by the same force, in the same place and at the same time. By this mechanism the oil globules are,

as soon as formed, coated with a liquid soap which a moment later hardens about them in the form of soap membranes. These soap membranes at the moment of their formation are not as capable of holding the globules as they are later, when, on cooling, they become more resisting. If this theory be true, it would follow that an appreciable length of time must elapse after the formation of an emulsion before it reaches its highest degree of stability. And this, in fact, I find to be true, that the emulsions can be more easily destroyed at the moment of their formation than later, and it is only in explanation of this and other facts that the above theory is offered. The following conclusions I draw from my experiments, and some of them are best explained by this theory.

1. If bile be present an emulsion cannot form, although all the conditions otherwise favorable to its formation be present. This fact was pointed out by Gad, and he offered in explanation that the soap-dissolving properties of the bile prevented the formation of insoluble soap membranes, and that the unprotected oil globules ran together and came to the surface as free oil.

2. If bile is added to an emulsion, the moment after it is formed the emulsion rapidly clears by creaming, but no free oil appears on the surface. Here it seems that the soap not in membranes is dissolved. This increases the specific gravity and diminishes the viscosity of the liquid, and as a result the soap-coated globules rise to the surface as cream; why it is that the soap in the membranes more quickly acquires the property of resisting the solvent action of bile than the soap not in membranes I cannot say, yet this seems the only explanation of the above phenomenon.

3. If bile be added to an emulsion some minutes after it has formed, it has no effect in destroying the emulsion. The above propositions clearly indicate that an appreciable length of time must elapse after the formation of an emulsion before it reaches its highest degree of stability.

4. One-tenth per cent. nitric and sulphuric acid and $1/5$ per cent. lactic acid solutions rapidly destroy emulsions, the free oil running to the surface. Acids destroy emulsions by combining with the base of soaps and freeing the fatty acids; the soap being thus destroyed, the liquid is much less viscous, while the specific gravity is very little altered. The oil globules are

therefore driven to the surface as cream, but if the acid be stronger, the soap in membrane is also destroyed, and free oil floats on the surface. The membrane soap is here found to be more resisting to soap destroyers than soap not in membranes.

5. Hydrochloric acid has a much less destructive influence on emulsions than has nitric or sulphuric acid, and lactic acid has a less destructive influence than acetic.

6. If sapo medicatus[1] be shaken in a $^1/_{10}$ per cent. nitric or sulphuric acid solution the soda of the soap will combine with the nitric or sulphuric acid and fine globules of free fatty acid will rise to the surface. Sapo medicatus is more easily destroyed by nitric and sulphuric acids than it is by hydrochloric acid. These facts strongly corroborate the opinion that acids destroy emulsions by destroying soaps.

THE FAT-SPLITTING PROPERTIES OF PANCREATIC JUICE.

Since the publications[2] of Claude Bernard, physiologists have generally believed that pancreatic juice has the property of splitting neutral fats into fatty acid and glycerine. Claude Bernard himself believed that the pancreatic juice had a twofold action on fats. In the first place, he said that when neutral oil and pancreatic juice were shaken together an instantaneous emulsion resulted. In the second place, that the prolonged action of pancreatic juice on neutral oil would develop fatty acid. He did not in any way associate these two processes, and believed them to be due to entirely different properties of the juice, the emulsion being an instantaneous process and the fat splitting occurring only after considerable time. And these two processes are still described as separate and distinct properties of pancreatic juice in some of our most recent text-books. But since the publications of Brucke and Gad, most German physiologists have associated these processes, believing that the emulsion was wholly due to the fatty acid which had been developed in the oil by the fat-splitting ferment, and that the pancreatic juice contained no emulsion ferment; this opinion was a matter of inference from the works of Brucke, Gad and others, rather than from actual

1 A soda soap made with olive oil acids.

2 *Compt. rend. de l'acad. de Paris*, T. xxviii. *Arch. général*, 1849. *Mémoire sur le Pancreas*, Paris, 1856.

experiments with the juice itself. I have failed to find that any systematic work in this direction had been done with pancreatic juice since the days of Claude Bernard. Quite a number of attempts have been made, but the difficulties in obtaining a normal juice were so great that no extensive work has been done and no important fact added to our knowledge. But while almost no work has been done with the juice itself, an immense amount of work has been done with pancreatic extracts and infusions made from the gland. Physiologists have seemed to take for granted that, in studying the physiological properties of pancreatic juice, the juice itself offered no advantage over these extracts. In fact, they seemed to believe from the great difficulty in obtaining a normal juice that the extracts were preferable, and our knowledge of the present day is based almost exclusively on experiments with the extracts, and but for the fact that they contain a fat-splitting ferment the time-honored opinion of Claude Bernard would have carried but little weight. For these reasons, therefore, a systematic investigation into the fat-splitting properties of the pancreatic juice seemed to offer a fertile field for work.

Although in the beginning the obstacle of obtaining normal juice in sufficient quantities to prosecute this investigation seemed insurmountable, yet I was fortunate enough to hit upon a method by which I could readily obtain from the rabbit a normal juice in sufficient quantities for experimental purposes. The operation for temporary pancreatic fistula in the rabbit is easily and quickly done as follows. Make an abdominal incision in the linea alba two and one-half inches long. Bring the duodenum, which is readily found high up in the right hypochondriac region, through this opening, run down the gut to a point where the peritoneum binds it so closely that it will not come through the opening, and just at this point will be found the pancreatic duct as it runs through a leaf of the pancreas to the small intestine. Resect two inches of the intestine at this point, leaving its mesenteric attachment, tie the cut ends of the intestine above and below and drop them in the cavity, bringing the resected portion through the abdominal wound. The abdominal wound is now partially closed by stitches, leaving only sufficient opening for the mesentery running to the resected gut. This resected gut is now laid open opposite the mesenteric attachment and spread out on the abdominal wall. The ends of the gut are clamped and its mar-

gins packed with absorbent cotton to prevent bleeding. Insert a small glass cannula through the pancreatic papilla into the pancreatic duct and cover the exposed mucous membrane with absorbent cotton saturated with common salt solution. The flow of juice begins at once and continues from four to six hours. In this manner about 1 c.c. of juice uniform and powerful in physiological action may be collected. This operation is a modification of the Heidenhain permanent fistula operation[1] and has the advantage of being simple and uniformly successful.

In my experiments I used the pancreatic juice of the rabbit, as it seemed quite impossible for me to obtain from the dog a normal juice in sufficient quantities for experimentation. The fat used was neutral olive oil.

I worked for several weeks with very faulty methods before I hit upon the method which I afterwards used, and which, I think, is admirably adapted to the study of the fat-splitting properties of pancreatic juice. The foundation-stone of the method is the spontaneous emulsion method of Gad. We have previously seen how by this method we may determine when an oil has the proper percentage of fatty acid to give the best spontaneous emulsion under certain given conditions. After having established the conditions under which one can get a good emulsion with a certain per cent. ($5\frac{1}{2}$) of fatty acid, it is evident that we can use this method for determining when an oil has this percentage of fatty acid, and since the completeness of the spontaneous emulsion will be in direct proportion to the nearness with which the quantity of fatty acid in the oil approaches this percentage, we have also a method of estimating the amount of increase of fatty acid in any oil by testing its spontaneous emulsibility from time to time. For example, let us suppose that we have a neutral oil in which fatty acid begins to develop, and that this process slowly continues until all the oil is changed into fatty acid and glycerine. If the test of spontaneous emulsibility be applied to such an oil by placing a drop of it from time to time on carbonate of sodium solution, we get at first no emulsion at all, and then with the development of some fatty acid a slight emulsion, then more and more with increasing quantities of acid until the maximum emulsion is reached, which indicates that about $5\frac{1}{2}$ per cent. of acid has been developed. The

1 *Handbuch der Physiologie*, Hermann, Bd. v.

emulsion then decreases with the further increase of acid until finally we get no spontaneous emulsion at all, which indicates about 12 per cent. of acid. Beyond this point the increase of acidity cannot be measured by spontaneous emulsion, but in this particular and under these circumstances the emulsion formed by shaking is of some value, for good emulsions may still be had in this way after too much acid has been developed for spontaneous emulsion. But the greater the amount of acid the more shaking is required to give a good emulsion, until finally when all the oil has been changed into fatty acid and glycerine we get no emulsion at all, but only a cloudiness due to the insoluble soap formed. In this method we have a simple means of approximately estimating the increase of fatty acid in an oil and of determining when all the oil has been changed to acid and glycerine. This method is not used to determine the exact quantity of acid which an oil contains, but is used rather to make a comparative estimate of the amount of acid in the same oil at different times and in different oils at the same time.

This method is applied to the study of the fat-splitting properties of pancreatic juice in the following manner. Arrange a series of watch-glasses containing a $\frac{1}{4}$ per cent. solution of carbonate of sodium. Take a small test-tube of 2 c.c. capacity and place in it $\frac{1}{8}$ c.c. of pancreatic juice and twice as much neutral olive oil. Shake the tube and allow the juice and oil to separate, then pipette a drop of oil from the surface and place it on the soda solution in watch-glass 1. Again, shake the tube and allow the oil and juice to separate, then pipette as before, placing a drop of oil in watch-glass 2. Again shake and pipette as before, and repeat this process every three or four minutes until the experiment is completed. The beginning of the experiment and the time of each pipetting must be carefully noted. If the pipettings are three minutes apart, then the first drops of oil will have been exposed three minutes to the action of pancreatic juice, the second drop six minutes, the third drop nine minutes, and so on. By the amount of spontaneous emulsion occurring in these drops when placed on the soda solution one can comparatively estimate the quantity of fatty acid they contain. For example, in an experiment such as I have just narrated one may find very little emulsion in glass 1, more in 2, a fair emulsion in 3, good in 4, and the maximum in 5, and then the emulsion gradually decreases.

By such experiments as this the fat-splitting properties of pancreatic juice can be beautifully demonstrated, and an idea formed of the rapidity of its action. There is a possible element of error in this method which had better be spoken of here. It would seem that the alkali of the pancreatic juice would combine with the fatty acids, forming soap, and in this way the oil would soon be emulsified in the juice itself and not separate after shaking. This would indeed be a serious drawback if it actually occurred, but in truth it does not occur until late in the experiment, after we have obtained the information we sought by the spontaneous emulsion method. It is true that after a large quantity of acid has developed and by repeated shaking we get an emulsion of oil in the juice which somewhat interferes with the method. Although the sodium in the pancreatic juice exists in the form of a carbonate, it seems to be peculiarly associated with some other substance which interferes with its combining with fatty acid in the formation of soaps. This may be illustrated by the following interesting experiment. Place in a small test-tube drawn out like a pipette equal quantities of pancreatic juice and neutral olive oil, $\frac{1}{2}$ c.c. each. Shake the tube and set aside for twenty-four hours. At the expiration of this time break the pipette point and allow the contents of the tube to escape slowly through the opening thus formed in the bottom of the tube. The pancreatic juice, being at the bottom, is the first to escape, and it is clear and strongly alkaline; then comes the oil which formed the upper layer, and it is strongly acid. Here we have a rancid oil and an alkaline fluid in contact for twenty-four hours with very little soap formation. This experiment clearly indicates that something interferes with the formation of soap from the alkalies of the pancreatic juice. This is a plausible explanation of why the element of error caused by soap formation does not interfere with the practical application of the method. But even the small element of error which is introduced by soap formation may be reduced to a minimum by using small quantities of juice and three or four times as much oil, and in that way the quantity of soda is greatly reduced and the action of the juice is but slightly retarded. This latter seems a strange statement, yet I have found in my experiments that within the limits named, the same quantity of juice splits large quantities of oil almost as readily as small. In passing, let me again call

attention to the experiment above narrated as a simple and striking lecture experiment. The alkalinity of the juice and the acidity of the oil as it follows through the same opening may be demonstrated by litmus paper or solution. With these details as to method we are prepared to consider pancreatic juice and its action on neutral fats.

1. The pancreatic juice of the rabbit is alkaline and remains so for some time after it is removed. On two occasions I tested juice that had stood exposed at room temperature for twenty-four hours and found it alkaline and physiologically active. Different specimens of pancreatic juice may vary in physiological activity. As a rule, the juice obtained from a fistula that has been acting several hours is not as active as juice from the same fistula obtained soon after the operation.

2. If pancreatic juice be shaken with neutral olive oil, the oil rapidly takes on an acid reaction. That this acidity is due to fatty acid is shown by the facts that all the acid may be extracted with ether and the oil made emulsible by its presence. The gradual yet rapid development of fatty acid by the action of pancreatic juice on neutral olive oil may be beautifully demonstrated by pipetting drops of oil at intervals from the surface of a mixture of pancreatic juice and neutral olive oil and placing them on a $\frac{1}{4}$ per cent. solution of carbonate of sodium in a series of watch-glasses. Soon we have a slight emulsion, then more and more until the maximum is reached, then the amount of emulsion becomes less and less as too much fatty acid is developed, until finally we have no spontaneous emulsion at all. That an excess of fatty acid is the cause of the decrease and cessation of spontaneous emulsion may be demonstrated as follows. Take a drop of oil from a mixture of oil and pancreatic juice after it has passed the limits of spontaneous emulsibility and mix it with neutral olive oil, and the mixture is spontaneously emulsible. In one experiment, for example, I took one drop of oil that had passed the stage of spontaneous emulsibility and mixed it with four drops of neutral olive oil, and one drop of the mixture on soda solution gave a beautiful spontaneous emulsion. Here one drop of the oil acted on by the juice contained sufficient fatty acid to make five drops of oil spontaneously emulsible, that is, to give five drops of oil about $5\frac{1}{2}$ per cent. of fatty acid. The drop of oil acted on by the juice must therefore have contained

about 30 per cent. of fatty acid and the time required to develop it was thirty-five minutes. Since 30 per cent. of acid is so quickly developed, it seems a fair inference that the prolonged action of the juice would change all the oil into fatty acid and glycerine, and such, in fact, is found to be the case.

3. All the oil is split into fatty acid and glycerine by from one to two hours' action of the pancreatic juice—time varies with the specimen of the juice. This may be shown by pipetting such fatty matter from the surface of the juice and shaking it with soda solution and no emulsion will result, simply a little clouding, such as occurs when fatty acid is shaken with soda solution. But if one drop of this same fatty matter be mixed with six or eight drops of neutral olive oil, this mixture will, on being shaken with soda solution, give a good emulsion. This experiment is best performed by adding a small quantity of bile to the juice before adding the oil. The bile does not interfere with the fat-splitting action of the juice, but it does interfere with the formation of an emulsion, and for that reason the oil and juice continue to separate after shaking.

4. The time required for pancreatic juice, acting in glass tubes at room temperature, to develop sufficient fatty acid ($5\frac{1}{2}$ per cent.) in neutral olive oil to give the maximum spontaneous emulsion varies with different specimens of the juice and with the amount of shaking to which the juice and oil are subjected, but the average time as taken from my experiments was twenty minutes. In very active specimens of the juice it occurred as early as seven minutes, and in very poor specimens as late as sixty minutes. I also found that the juice did not act more rapidly in a basin of intestine than in the test-tubes. In these experiments the resected intestine containing the pancreatic papilla was held by a fenestrated quadrilateral clamp made for the purpose, and into the basin of the intestine thus formed the pancreatic juice would ooze. Neutral olive oil was dropped into this basin and mixed with the pancreatic juice, and this oil did not become spontaneously emulsible more quickly than the oil in the test-tubes, but the conditions here are also far from resembling those occurring in the normal duodenum, and the average rate of fat-splitting as established by these experiments is probably considerably below the rate at which fats are split in the duodenum. It is probable that the time required by the most

active juice more nearly represents the rapidity of action of pancreatic juice in the duodenum.

5. The action of the pancreatic juice on most of the fats is rapid and complete.

Castor oil is a notable exception to this rule, as only a very small quantity of acid is developed in it by the action of pancreatic juice for five hours at 37° C. Castor oil is therefore practically indigestible, and this may in part account for its cathartic action.

Pancreatic juice acts slowly on fats which have a melting point above body temperature, but it is an interesting physiological fact that their solidity at body temperature does not prevent their being split. Spermaceti, for example, the melting point of which is above 38° C., is slowly split by the action of the pancreatic juice.

6. As I have previously said, the pancreatic juice of the rabbit and neutral olive oil when shaken together show very slight tendency to the formation of an emulsion, and it is only after considerable acid has developed that repeated shaking will give a mixture resembling an imperfect emulsion. But if we mix and shake at intervals one part of neutral olive oil and one part of pancreatic juice for about fifteen minutes, and then add six parts of soda solution, we get at once an apparently good emulsion. This emulsion does not remain good; it always in the course of an hour or two clears by creaming, when the whole mixture will be found to have a strong acid reaction due to the large quantity of fatty acid developed. Whatever may be the explanation of the clearing of this pancreatic emulsion, the fact remains that an emulsion will form in the presence of pancreatic juice if carbonate of sodium solution be added, but it does not remain permanent.

7. A permanent pancreatic emulsion may be formed by pipetting the oil from the surface of a tube containing oil and juice and shaking it with the carbonate of sodium solution. The emulsion formed in this way remains very much the same for an indefinite length of time. In this experiment the oil is made emulsible by the action of the juice and is then separated from it and emulsified with the soda solution; the emulsion itself contains no pancreatic juice and therefore does not clear. This permanent pancreatic emulsion reacts to emulsion-destroying agents and soap dissolvers very like a fatty acid emulsion made

with rancid oil and sodium solution. For example, it is not destroyed by the addition of bile or fatty acids, but is destroyed by mineral acids, resisting hydrochloric better than nitric and sulphuric acids. The pancreatic emulsion also resembles the simple rancid oil emulsion in that an appreciable length of time must elapse after its formation before it reaches its greatest degree of stability. This may be demonstrated by adding bile in excess immediately after the formation of the emulsion, when it destroys the emulsion by creaming, but if the bile be added later no such effect is produced. It also resembles the rancid oil emulsion in that it cannot form at all in the presence of bile.

The most important application of the method I have described is in obtaining comparative information concerning the fat-splitting properties of pancreatic juice. This application of the method may best be explained by detailing an experiment inquiring into the difference in the rapidity of action of pancreatic juice at room (18° C.) and at body temperature (37° C.).

Arrange two rows of watch-glasses containing a ¼ per cent. carbonate of sodium solution. Take two small test-tubes, ⅛ c.c. of the same pancreatic juice in each, and to each tube add ⅛ c.c. of neutral olive oil. Shake both tubes equally and place one of them (A) in a sand bath kept in an oven at 37° C. and leave the other (B) at room temperature. At the expiration of three minutes pipette a drop of oil from A and place it in watch-glass 1, row 1; then as quickly as possible, with a clean pipette, take a drop from B and place it in watch-glass 1, row 2. Both tubes are shaken and replaced and at the expiration of three minutes a drop is again pipetted from the surface of each. That from A is placed in row 1, that from B in row 2. This process is repeated again and again to the end of the experiment. At the close of the experiment it will be found that the emulsion occurs almost twice as quickly in row 1 as in row 2. The three-minute drop of oil from A gives as good an emulsion as the six-minute drop of oil from B, and the nine-minute drop of oil from A gives the same emulsion as the eighteen-minute drop of oil from B. Since these tubes were, apart from the temperature, treated as nearly alike as possible, we infer that pancreatic juice acts about twice as rapidly at 37° C. as it does at 18° C. The average ratio of increased rapidity of action, taken from my experiments, was as one to one and eight-tenths.

Whatever objections may be urged against the absolute accuracy of the figures obtained by this method, the same do not apply to the comparative accuracy of these figures. Even though we may not be able by this method to estimate the amount of acid produced by pancreatic juice in nine minutes acting at 37° C., we do know by this method, whatever this amount may be, that it requires one and eight-tenths times as long for pancreatic juice to produce the same amount at 18° C. In comparative experiments such as this it is not necessary nor practicable to have an equal length of time between the pipettings, but it is important that the tubes should be shaken at as nearly the same time and pipetted at as nearly the same time as possible, so that the oil drops to be compared by spontaneous emulsibility may have been exposed to the action of the juice for the same length of time, thus establishing the comparative accuracy of the results.

The great value and wide application of this method is seen in the study of the influence of bile and other agents on the fat-splitting action of pancreatic juice.

Bile alone does not split fats. This seems a well-established physiological fact, which may be confirmed by shaking neutral olive oil and bile in a test-tube and pipetting the oil at intervals to the surface of a carbonate of sodium solution as in previous pancreatic experiments, when it will be found that oil shaken with bile for twenty-four hours does not become emulsible. The value of this method is here most conspicuous, as the emulsibility of the oil could not be tested in the presence of the bile, because the bile would prevent an emulsion even if the fatty acid had been developed. But in this method the oil is separated from the bile after they have been in contact twenty-four hours and its emulsibility tested, and in this point lies the great value and wide application of the method, since the very agents, such as bile and hydrochloric acid, which have the greatest influence on the fat-splitting action of pancreatic juice are the agents which interfere with the formation of emulsions.

Fresh rabbit bile removed from the gall-bladder was used in all my experiments.

In every comparative experiment the pancreatic juice which had been collected in a single tube was divided into two, three or four equal parts, according to the number of tubes used in the experiment. The bile was also shaken and divided just previous

to the experiment. In this way I could be reasonably sure that I was working with the same bile and same pancreatic juice in all the tubes.

By the methods described I reached the following conclusions:

1. An equal amount of fresh rabbit's bile will, on being added to rabbit's pancreatic juice, greatly hasten its fat-splitting action in the ratio of three and one-fifth to one. In experiments of this kind, tube A contains $\frac{1}{8}$ c.c. of pancreatic juice and $\frac{1}{2}$ c.c. of neutral olive oil, and tube B contains $\frac{1}{8}$ c.c. pancreatic juice and $\frac{1}{8}$ c.c. bile and $\frac{2}{8}$ c.c. of neutral olive oil. These tubes are treated alike and the emulsibility of the oil is tested from time to time as previously described. In this way the comparative rapidity with which fatty acid is developed in the oils may be determined. It is evident that in every experiment we can have two sets of figures from which to make our average, viz., the time required for the beginning and the time required for the maximum of spontaneous emulsion. In my general averages I have used both sets of figures, striking an average between them.

2. An equal quantity of a $\frac{1}{4}$ per cent. solution of hydrochloric acid will, on being added to pancreatic juice, retard its fat-splitting action in the ratio of two-thirds to one.

3. A mixture of equal quantities of bile and a $\frac{1}{4}$ per cent. hydrochloric acid solution will, on being added to pancreatic juice, greatly hasten its fat-splitting action in the ratio of four to one. The bile not only neutralizes the retarding influence of the hydrochloric acid on the fat-splitting properties of the juice, but it really acts more powerfully in hastening the action of the juice when in the presence of this acid than it does when acting alone. The contents of a series of tubes will best explain the class of experiments upon which this statement is based.

Tube A contains $\frac{1}{8}$ c.c. pancreatic juice and $\frac{2}{8}$ c.c. neutral olive oil. Tube B contains $\frac{1}{8}$ c.c. of pancreatic juice, $\frac{1}{8}$ c.c. of bile and $\frac{2}{8}$ c.c. neutral olive oil. Tube C contains $\frac{1}{8}$ c.c. of pancreatic juice, $\frac{1}{6}$ c.c. of bile, $\frac{1}{6}$ c.c. of a $\frac{1}{4}$ per cent. hydrochloric acid solution, and $\frac{2}{8}$ c.c. of neutral olive oil.

Three rows of watch-glasses containing soda solution having been arranged for the reception of the oil drops, the tubes are now shaken and pipetted as in previous experiments and the time and the result are carefully noted. In row 1 containing the oil drop from A, the emulsion begins in eight minutes, and

reaches the maximum in twenty minutes. In row 2 containing the oil from B, the emulsion begins in two and a half minutes and reaches the maximum in six and a quarter minutes. In row 3 containing the oil drop from C, the emulsion begins in two minutes and reaches the maximum in five minutes. These figures are the averages of a number of experiments.

4. If an equal quantity of a 3 per cent. solution of glycocholate of soda be mixed with pancreatic juice it hastens the fat-splitting action of the juice in the ratio of two and one-fifth to one.

5. A mixture of equal quantities of a 3 per cent. solution of glycocholate of soda and a ¼ per cent. solution of hydrochloric acid will, on being added in equal quantities to pancreatic juice, hasten its fat-splitting action in the ratio of two and one-third to one.

The glycocholate of soda solution, like the bile, not only neutralized the retarding influence of hydrochloric acid on the fat-splitting action of the juice, but it really acts more powerfully in hastening the action of the juice when in the presence of the acid than it does when acting alone. It must also be noted that the glycocholate of soda does not act as powerfully in hastening the fat-splitting action of the juice as the bile does. In the presence of bile the juice acts three and one-fifth times as rapidly as it does alone, and in the presence of a 3 per cent. solution of glycocholate of soda it acts two and a fifth times as rapidly. In the presence of bile and hydrochloric acid it acts four times as rapidly, and in the presence of glycocholate of soda and hydrochloric acid it acts two and four-fifths as rapidly. From this I infer that this property of the bile is chiefly but not wholly due to the glycocholate of soda it contains. The class of experiments by which these conclusions were reached is illustrated in Plate I,[1] which is in part reproduced from a photograph.

6. If one part of pancreatic juice be diluted with five parts of a ¼ per cent. carbonate of sodium solution its fat-splitting properties will be greatly retarded—in the ratio of one to eight—and further dilution with soda solution gives greater retardation, this property of the juice being practically destroyed when it is ten times diluted with this strength of soda solution. That this retarding influence is due to the soda, and not to the dilution, is

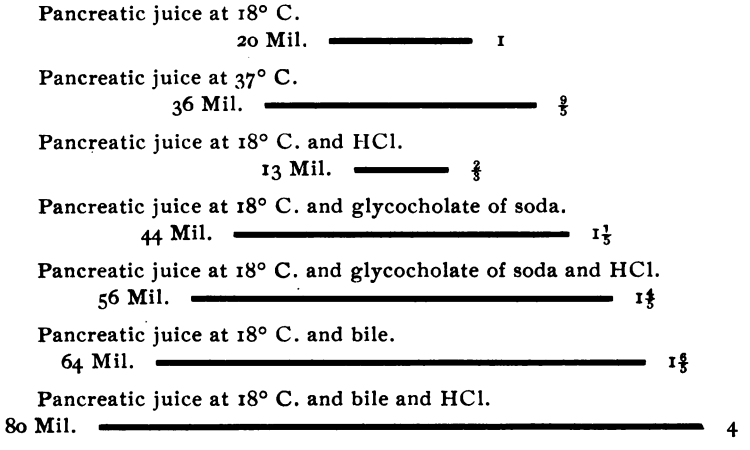
1 See original paper.

shown by the fact that if pancreatic juice be diluted with five parts of distilled water, its fat-splitting action is very slightly, if at all, retarded.

The retarding influence of soda solution may be shown by the same kind of experiments used to show the influence of bile, hydrochloric acid, etc., on the fat-splitting properties of pancreatic juice. But it seems possible that there might be considerable cause of error in this class of experiments, because of the presence of soda solution in one of the tubes. In an experiment of this kind, for example, one tube contains $\frac{1}{8}$ c.c. of pancreatic juice and $\frac{2}{8}$ c.c. of neutral olive oil, the other contains in addition to the same quantity of juice and oil $^5/_8$ c.c. of soda solution. In pipetting oil from the surface of two such tubes to test its spontaneous emulsibility, will not the result be greatly vitiated by the soda solution in one of the tubes, neutralizing the fatty acid as soon as formed? Theoretically this would seem to be an important source of error, but practically it is not of very great importance, since the results obtained by this method correspond closely to those obtained by another method which has not this source of error. The following experiment will illustrate this method. Take two small glass tubes. In one place $\frac{1}{8}$ c.c. of pancreatic juice and $\frac{1}{8}$ c.c. of neutral olive oil. Shake four or five minutes and add $^5/_8$ c.c. of soda solution and an immediate emulsion will result. To the other tube add $\frac{1}{8}$ c.c. pancreatic juice and $\frac{1}{8}$ c.c. neutral olive oil and $^5/_8$ c.c. of soda solution. Shake, and the emulsion will not appear for thirty or thirty-five minutes. In the first tube, the pancreatic juice acting alone on the neutral oil produced enough acid in four or five minutes to make the oil emulsible on shaking it with the soda solution. But in the tube 2, the presence of the soda solution retarded the action of the juice so that it required thirty minutes to produce sufficient fatty acid to give an emulsion. Carbonate of soda solution therefore retards the fat-splitting action of pancreatic juice in the ratio above given.

In the accompanying diagram I have taken a line twenty millimetres long to represent the working power of pancreatic juice acting alone at room temperature. The other lines represent the comparative working power of pancreatic juice under the conditions named, and were obtained from averaging all my experiments.

DIAGRAM SHOWING THE INFLUENCE OF BILE AND OTHER AGENTS ON THE FAT-SPLITTING PROPERTIES OF PANCREATIC JUICE.



The above diagram and accompanying figures are offered as the clearest and briefest manner of expressing the difference in the rapidity of action of the various mixtures. It is not even hoped that these figures are absolutely correct, but it is my belief that relatively they are approximately correct, and therefore have an all-important bearing on the pancreatic digestion of fats. We may summarize:

1. Pancreatic juice can, acting alone, do a certain piece of work in x minutes, viz., develop in neutral olive oil a sufficient quantity of fatty acid to give the best spontaneous emulsion.

2. Pancreatic juice acting in the presence of five parts of a $\frac{1}{4}$ per cent. carbonate of soda solution will require $8x$ minutes to do the same work, and in the presence of ten parts of the same solution its action will be almost destroyed.

3. Pancreatic juice acting in the presence of an equal quantity of a $\frac{1}{4}$ per cent. solution of hydrochloric acid will require $^3/_2x$ minutes to do the same work.

4. Pancreatic juice acting in the presence of an equal quantity of a mixture of bile and a $\frac{1}{4}$ per cent. hydrochloric acid solution will require only $\frac{1}{4}x$ minutes to do the same work.

From the last two propositions it would follow that, if bile be added to pancreatic juice which is acting in the presence of

hydrochloric acid, the fat-splitting action of the juice will be hastened as $^3/_2$ to $\frac{1}{4}$ or as six to one, and reversely, that if the bile be withdrawn or cut off from pancreatic juice which has previously been acting in the presence of both bile and hydrochloric acid, the fat-splitting properties of the juice will be retarded as six to one.

APPLICATION OF THESE PRINCIPLES TO THE INTESTINAL DIGESTION OF FATS.

It is needless to say that my experiments were planned with the idea of placing pancreatic juice under conditions as nearly as possible resembling those under which it acts in the intestine. The influence of a $\frac{1}{4}$ per cent. solution of HCl was studied because of the presence of this acid in the duodenum, where the pancreatic juice comes in contact with the fats. The influence of bile and of a mixture of bile and hydrochloric acid were studied for the same reason. The influence of dilution with a $\frac{1}{4}$ per cent. solution of carbonate of sodium was studied because it was thought, that, as the pancreatic juice passed downward into the small intestine, it might be subjected to some such influence, since the succus entericus contained this percentage of carbonate of soda. The conclusions therefore to which I have arrived must, if true, have a very important bearing in the explanation of the intestinal digestion of fats. I infer from my experiments that in the duodenum the mixture of bile and hydrochloric acid furnishes the best known conditions for expediting the fat-splitting action of pancreatic juice, and the cutting off of the bile would retard the fat-splitting action of the juice six times. It may also be of some physiological importance to note that the agents bile and HCl which expedite the fat-splitting absolutely preclude the formation of emulsions. The duodenum therefore offers the most favorable conditions for the splitting of the fats and the most unfavorable for their emulsification. In the jejunum and ileum these conditions seem to be exactly reversed. The intestinal juice containing, as it does, $\frac{1}{4}$ per cent. of carbonate of soda, would not only furnish the conditions for the spontaneous emulsification of the rancid fats, but would also retard the fat-splitting action of the pancreatic juice. I do not wish to express the belief that intestinal juice plays just such a rôle as this in the intestinal digestions of fats, but only

offer it as a deduction from test-tube experiments, thinking it may have some physiological bearing.

From my experiments I infer that pancreatic juice must act very rapidly under the favorable conditions found in the duodenum. In some of my experiments at room temperature, good specimens of pancreatic juice, aided by the presence of bile and hydrochloric acid, produced, in neutral olive oil, 5½ per cent. of fatty acid in two minutes. At body temperature this work would have been accomplished in one minute, and under the favorable conditions offered by the duodenum it would probably have been done in even less time.

This rapidity of action of pancreatic juice is of great physiological importance, since it is evident that at this rate all the fats would be split into fatty acid and glycerine in the time required for intestinal digestion, unless this action of the juice was checked or retarded in some manner.

IMPORTANCE OF BILE IN THE INTESTINAL DIGESTION OF FATS.

The various conditions which have an influence on the intestinal digestion of fats have been developed by natural selection, and so far as we know they are the best for the purposes they serve. The comparative immobility of the duodenum, its close attachment to the head of the pancreas, its horse-shoe shape, all, no doubt, have an influence on the rate of passage of food-stuffs. This rate, which is chiefly controlled by these and other anatomical conditions, was established to accord with normal digestive functions, and by this mechanism the fats are exposed to the action of pancreatic juice just long enough to allow for whatever action that juice may have in fat digestion. Let us suppose that under normal conditions the fats are exposed in the duodenum to the action of pancreatic juice for x minutes, and that this time is just sufficient to allow for whatever fat-splitting is necessary at this point. Now, if the bile be cut off, the rate of passage of the food-stuffs, which is chiefly controlled by anatomical conditions, remaining the same, the fat would still be exposed to the action of the juice for only x minutes. But since in the absence of the bile the pancreatic juice is able to accomplish only one-sixth of the fat-splitting which it normally does, it would follow that the fats would pass with only one-sixth of the amount of splitting

that normally occurs, and since the splitting of the fat is, as recognized by all physiologists, a necessary preliminary step in fat digestion, it would follow that the fats would pass in great part undigested. This gives to bile a most important and definite position among the juices which assist in fat digestion, since we have here pointed out at least one of the ways in which it exerts its wonderful influence in fat digestion. Physiologists have been led to believe, through much clinical and experimental[1] evidence, that the bile was necessary to fat digestion. How and where it acted has been one of the greatest of physiological mysteries. The experiments of Westinghausen[2] seemed to show that bile promoted the passage of the fats through membranes, and this was thought by some physiologists to have a bearing on the absorption of fats. But since the publication of Groeper[3] denying that bile had any such action we have been quite as much at sea as ever in explaining the action of bile in fat digestion.

I wish to thank Professor Gad for his kindness and advice during the prosecution of these studies.

1 Of special interest are the recent experiments of A. Dastre in the *Arch. de Physiologie et Pathologie*, Paris.

2 *Archiv f. Anat. u. Phys.*, 1873.

3 *Archiv f. Anat. u. Phys.*, 1889.

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II.—FAT-DIGESTION.

During the spring and summer of 1890, in the Berlin Physiological Laboratory, I made a study of the fat-splitting properties of pancreatic juice. I read a paper on this subject before the physiological section of the Tenth International Medical Congress, and published a detailed account of my work in the *Journal of Physiology.* (*a*)[1]

It is my purpose in the present paper to show what application my published experiments (*a*) may have in explaining fat-digestion. That I may do this intelligently, it will be necessary to review our present knowledge of this subject.

C. Bernard believed that pancreatic juice had a twofold action on fats. In the first place, he said that when neutral oil and pancreatic juice were shaken together an instantaneous emulsion resulted, and in the second place, that the prolonged action of pancreatic juice on neutral oil would develop fatty acid. He did not in any way associate these two processes, and believed them to be due to entirely different properties of the juice; the emulsion being an instantaneous process and the fat-splitting occurring only after considerable time. And these two processes are described as separate and distinct properties of pancreatic juice in some of our most recent text-books. But some of the more recent German books have, upon the observations of Brucke and Gad, taught the relationship of these processes. From the fact that the addition of fatty acid to neutral oil makes the mixture readily emulsible in an alkaline liquid, they infer that the emulsion of fats in pancreatic juice, as observed by Bernard, is not due to an emulsion ferment, but is rather due to the development of fatty acid in the fat by the action of the pancreatic juice. They believe, therefore, that the first and only specific action of

1 Cambridge, Eng., April, 1891. The mark (*a*), which occurs frequently in the text, always refers to my published experiments in the *Journal of Physiology.*

pancreatic juice on fats is the splitting of them into fatty acid and glycerine, and that by reason of this an emulsion results in the alkaline pancreatic juice; but this opinion is a matter of inference from the work of Brucke and Gad rather than from actual experiment with the juice itself. The special emulsion ferment of Bernard, although not disproven, is not believed in by many German physiologists. But physiologists very generally believe that the alkalinity of pancreatic juice promotes the emulsification of the rancid fats, and this latter opinion I have in my published experiments (*a*) shown to be erroneous. We may, therefore, sum up our present knowledge of the action of pancreatic juice on fats in very few words, viz.: it splits neutral fats into fatty acid and glycerine. We know nothing about the rapidity nor the influence of other agencies on this action.

Let us now note our present knowledge of the action of bile in fat-digestion. The part that bile plays in intestinal digestion seems one of the most obscure of physiological problems.

We know that it has a very decided influence in fat-digestion; this is proven by a large amount of clinical and experimental testimony. The records of clinical medicine teem with cases showing that in occlusion of the bile-ducts the fats are imperfectly digested, and experimental physiology, by ligating the bile-ducts in animals, has amply confirmed this observation. A. Dastre[1] found that when the bile-duct of the dog was ligated and the bile turned into the intestinal canal, midway between the stomach and cæcum, by making a fistula between gall-bladder and intestine at this point, the chyle-vessels remained transparent throughout that part of the intestine between the stomach and the entrance of the bile, and only became milky 15 cm. below the point where the bile was turned in.

All of this testimony only gives us the indefinite knowledge that bile has some important influence in fat-digestion, but does not give us the slightest clue to the manner in which it exerts its wonderful influence.

We do not know whether it assists in splitting the fats, or in emulsifying them, or in promoting their absorption, or whether it acts in some other mysterious manner; and the contradictory statements of Westinghausen[2] and Groeper,[3] concerning the ques-

1 *Arch. of Phys. and Pathol.*, Paris, 1890.

2 *Archiv f. Anat. u. Phys.*, 1873. 3 Ibid., 1879.

tion whether or not bile promotes the passage of fats through animal membranes, have left us entirely at sea on this important point. In fact, our only definite knowledge concerning the action of bile in intestinal digestion is that it prevents putrefaction, but what influence this anti-putrefactive action of bile may have on fat-digestion we do not know. We may, therefore, say that we are entirely ignorant of the manner in which bile acts in promoting the intestinal digestion of fats.

One fact is quite well established in the physiology of fat-digestion, viz., that splitting of fats is an important, if not necessary, preliminary step in fat-digestion. But while physiologists agree that the splitting of fats is an important factor in their digestion, there is a great difference of opinion as to the extent of the splitting required—some believing that only sufficient acid is developed in the fat to make it emulsible in an alkaline liquid, and others that all the fat must be split into fatty acid and glycerine before it can be absorbed. With this introduction we will now note the conclusions which I have reached by my experiments (*a*), and then show what application they may have in fat-digestion:

1. Heating neutral fats will develop in them fatty acid, and, therefore, make them emulsible in an alkaline liquid. The cooking of fats will, therefore, by developing in them fatty acids, assist in their digestion.

2. Pancreatic juice has the property of rapidly splitting neutral fat into fatty acids and glycerine. This action is so rapid that it may develop 5½ per cent. of fatty acid in seven minutes, and all the fat may be split into fatty acids and glycerine in less than an hour. These figure are taken from pancreatic juice acting in test-tubes at room temperature.

3. Pancreatic juice splits fats almost twice as rapidly at body (37° C.) as at room (18° C.) temperature. It can, therefore, develop in neutral olive oil 5½ per cent. of fatty acid in four minutes, and split all the oil in half an hour.

4. Pancreatic juice of the rabbit does not contain an emulsion ferment; it does not even furnish good conditions for giving an emulsion with oil containing fatty acid.

5. The presence of bile or of a ¼ per cent. solution of hydrochloric acid, or of both, not only forbids the formation of emulsions, but they also exercise a destructive influence on newly formed emulsions.

6. An equal amount of fresh rabbit's bile will, on being added to rabbit's pancreatic juice, greatly hasten its fat-splitting action in the ratio of three and one-fifth to one.

7. An equal quantity of a ¼ per cent. solution of hydrochloric acid will, on being added to pancreatic juice, retard its fat-splitting action in the ratio of two-thirds to one.

8. A mixture of equal quantities of bile and a ¼ per cent. hydrochloric acid solution will, on being added in equal quantities to pancreatic juice, greatly hasten its fat-splitting action in the ratio of four to one. The bile not only neutralizes the retarding influence of the hydrochloric acid on the fat-splitting properties of the juice, but it really acts more powerfully in hastening the action of the juice when in the presence of this acid than it does when acting alone.

9. If one part of pancreatic juice be diluted with five parts of a ¼ per cent. carbonate of sodium solution, its fat-splitting properties will be greatly retarded in the ratio of one to eight, and further dilution with soda solution gives greater retardation; this property of the juice being practically destroyed when it is ten times diluted with soda solution.

We may summarize from the above propositions as follows:

a. Pancreatic juice can, acting alone, do a certain piece of work in x minutes, viz., develop in neutral olive oil a certain quantity of fatty acid.

b. Pancreatic juice acting in the presence of five parts of a ¼ per cent. solution of sodium carbonate will require eight x minutes to do the same work, and in the presence of ten parts of the same soda solution its action will be almost destroyed.

c. Pancreatic juice acting in the presence of an equal quantity of a ¼ per cent. solution of hydrochloric acid will require $^3/_2\ x$ minutes to do the same work.

d. Pancreatic juice acting in the presence of an equal quantity of a mixture of bile and a ¼ per cent. hydrochloric acid solution will require only $\frac{1}{4}\ x$ minutes to do the same work.

From the last two propositions we may make another:

e. If bile be added to pancreatic juice which is acting in the presence of HCl, the fat-splitting action of the juice will be hastened as one and one-half to one-fourth, or as six to one; and reversely, if the bile be cut off from pancreatic juice which has previously been acting in the presence of both bile and hydro-

chloric acid, the fat-splitting properties of the juice will be retarded as six to one.

APPLICATION OF THESE PRINCIPLES TO FAT-DIGESTION.

During the process of cooking, as we have seen (*a*), a conconsiderable percentage of fatty acid is developed in fat, so that the fat in the food passes into the stomach not as neutral, but as rancid[1] fat. In the stomach, so far as we know, the rancidity of the fat is not increased, but it is mixed with hydrochloric acid, and, therefore, passes into the duodenum very much increased in acidity. The contents of the stomach, as it passes into the duodenum, contained ¼ per cent. of hydrochloric acid. The fat, therefore, enters the duodenum as part of such an acid mixture, and in such a state is subjected to the action of pancreatic juice.

Pancreatic juice has the property of rapidly splitting neutral fat into fatty acid and glycerine; acting alone at room temperature it can develop in neutral olive oil 5½ per cent. of fatty acid in seven minutes, and at body temperature it can develop this amount of acid in four minutes, and split all the oil in about half an hour. This indicates the rapidity of action of pure pancreatic juice on neutral olive oil, but such are not the conditions found in the duodenum. The fat in the duodenum is not subjected to the action of unadulterated pancreatic juice, but to pancreatic juice mixed with bile. The bile and pancreatic juice are poured through a common opening into the duodenum, and are, therefore, mixed before they come in contact with the fat. Of great physiological importance, therefore, is the conclusion to which I have arrived—that an equal quantity of bile will hasten the fat-splitting action of pancreatic juice as three and one-fifth to one. The preliminary mixing of bile with pancreatic juice enables the juice to do three and one-fifth times the work it would otherwise do. It is a fact here worthy of note, that we have in this expediting action of bile on the fat-splitting properties of pancreatic juice at least one of the physiological reasons for the union of the bile and the pancreatic ducts in the carnivora. It may be well here to refer to the fact that bile alone does not split fats (*a*). Its action, therefore, in assisting pancreatic juice is purely indirect, and is, therefore, the more remarkable.

We have in the above an indication of the rapidity of action

1 Rancid fat means fat containing fatty acid.

of a mixture of bile and pancreatic juice on pure neutral olive oil. But such are not the conditions found in the duodenum, where, as we have above stated, the fats are mixed with a ¼ per cent. solution of hydrochloric acid. We must, therefore, see what influence a mixture of bile and hydrochloric acid will have on the fat-splitting properties of pancreatic juice, for it is in the presence of such a mixture as this that the juice must act in the normal duodenum. Of great physiological importance, therefore, is the conclusion to which I have arrived by test-tube experiments (*a*), that a mixture of bile and hydrochloric acid hastens the fat-splitting action of pancreatic juice as four to one. If pancreatic juice can, as stated above, develop 5½ per cent. of fatty acid in four minutes, then the same juice can in the presence of bile and hydrochloric acid do the same work in one minute. Again, if pancreatic juice can, acting alone, split all the oil into acid and glycerine in thirty minutes, then in the presence of bile and hydrochloric acid it can do the same work in about eight minutes.

The above figures are taken from test-tube experiments (*a*), and the fat used was neutral olive oil. Now, as I have shown elsewhere (*a*), olive oil is very easy of decomposition. The fat in the food is, therefore, not, on the average, as readily decomposed as olive oil, and there must necessarily be many other sources of error in attempting to imitate the conditions found in the intestinal canal.

The above figures, therefore, are thought to be only relatively and approximately correct, but it is my belief that they are sufficiently accurate to indicate that the agencies acting in the healthy duodenum furnish the very best known conditions for hastening the fat-splitting properties of pancreatic juice, and chief among these agencies are: the body temperature, the presence of bile and hydrochloric acid, and the peristaltic movements of the duodenum, which insure the mixing of its contents.

That fats are split with great rapidity under the very favorable conditions furnished by the duodenum is a physiological fact of great importance, for it is quite evident that if this rate of fat-splitting be continued as the food passes down the small intestine, all the fat would be split into acid and glycerine long before the period required for intestinal digestion. One of two things must therefore occur—either all the fat is split, or some agency exercises a retarding influence on the fat-splitting process as the food passes down through the intestine; but I shall have more to

say on this point when we come to study the changes that occur in fat after it leaves the duodenum.

Let us now inquire what influence the conditions furnished by the healthy duodenum would have on the formation of emulsions. I have shown by my experiments (*a*) that pancreatic juice not only does not contain an emulsion ferment, but that it does not even furnish good conditions for giving an emulsion with oil containing fatty acid. The alkali in the juice seems to be in some combination which does not readily allow it to combine with the fatty acid to form a soap, and this we have seen (*a*) is a necessary preliminary step in the formation of a permanent emulsion. But the most important fact bearing on the formation of emulsions in the duodenum is that the agents, bile and hydrochloric acid, the most important in furnishing the favorable conditions for the splitting of fats, are the very agents which forbid the formation of an emulsion. An emulsion not only cannot form in the presence of bile and hydrochloric acid, but these agents actually destroy newly formed emulsions (*a*). It seems, therefore, that the conditions furnished by the normal duodenum are as admirably adapted to prevent emulsion-forming, as they are to promote fat-splitting. We may, therefore, in a few words, sum up the changes occurring in fats in the duodenum, viz.: fats are rapidly split, but not emulsified, in the normal duodenum.

The rancid fat leaves the duodenum, and passes into the jejunum, and on downward through the small intestine; there it comes in contact with the intestinal juice and other agents which have an influence on its digestion.

But, first, let us note certain anatomical conditions which may have an influence in fat-digestion. The horseshoe shape and the comparative immobility of the duodenum will make the rate of passage of food-stuffs through it comparatively slow, and in that way contribute to the fat-splitting by exposing for a longer time the fats to the fat-splitting agencies acting under the most favorable conditions. But as the fats leave the duodenum, they pass at once into the larger, and more freely movable, descending jejunum, and their rate of passage through this portion of the intestine would therefore be much more rapid than through the duodenum, and the influence of the bile and hydrochloric acid on the fat-splitting properties of pancreatic juice would be rapidly lost in dilution with the intestinal contents. These anatomical

conditions have much more significance when considered in the light of my observation (*a*) that five parts of a ¼ per cent. carbonate of soda solution will greatly retard the fat-splitting properties of pancreatic juice in the ratio of one to eight, and that ten parts practically destroy this property; since we infer from this that intestinal juice which contains this percentage of soda will have the same retarding influence. This retarding influence of the intestinal juice begins as soon as the food leaves the duodenum, and is the more rapidly felt the more rapidly the food is hurried along the jejunum. It would seem, therefore, that the conditions in the small intestine below the duodenum are most unfavorable to fat-splitting, and it is probable that they very greatly retard, if they do not entirely check, the fat-splitting process.

Let us now consider what influence the conditions found in the jejunum and ileum may have on the formation of emulsions, and in considering this question it will be necessary to keep constantly in mind one important fact, viz.: when rancid fat is mixed with a ¼ per cent. carbonate of soda solution, a good, permanent emulsion always results (*a*).

The intestinal mucous membrane of the jejunum and ileum is, during digestion, constantly bathed with intestinal juice, which we have before stated contains about ¼ per cent. of carbonate of soda. The rancid fat passing along the intestine must, in being absorbed, come in contact with this ¼ per cent. solution of carbonate of soda; this being the case, more or less emulsion would necessarily result, this emulsion occurring just at the mucous surface, and probably just prior to resorption. This seems to me to be an inevitable conclusion from the conditions existing in the small intestine. This explanation does not comprehend the emulsification of all the fats in the small intestine, but only such a part of it as, in the process of resorption, passes through the alkaline coating on the mucous membrane. That the fat in the lumen of the small intestine is often not emulsified, and that the intestinal contents are sometimes acid, argues nothing against the above application of existing facts. I do not wish to express the belief, however, that the emulsification of fats is necessary to their resorption, for this I do not believe. A portion of the fat may pass into the villi in the form of soluble soap. This is, in fact, very probable, since in the conditions above named, soap-formation is the chemical force which produces the emulsion.

Where the intestinal contents are very rancid, it is quite probable that soap-formation, independent of any influence it may have in forming emulsions, is an important preliminary step to fat-resorption. All I wish to insist upon is that the conditions found in the intestinal canal clearly indicate that emulsion-forming is also one of the preliminary steps to fat-resorption, and I may add that to my mind it is the most important step.

Milk is a physiological emulsion that is absorbed when thrown into the rectum, and may be taken and digested by animals in which both bile and pancreatic ducts have been ligated. This is sufficient proof that the emulsion of the fat in milk is an important preliminary step to its resorption, and would lead us to infer that the proper emulsion of the fats in the intestine would in like manner promote their absorption. But it is not the purpose of this paper to enter further into the subject of fat-resorption. Having traced the fats through the various stages of their digestion until they are almost, if not quite, prepared for resorption, we can return to the consideration of certain interesting and practical questions, which were omitted because they had no direct bearing on the question under consideration.

That hydrochloric acid retards the fat-splitting properties of pancreatic juice is a fact of considerable clinical and pathological importance, since this retarding influence would be felt when the bile was shut off from the intestinal canal by occlusion of the bile-ducts from any pathological cause. We have elsewhere (*a*) shown that this retarding influence of hydrochloric acid may be represented by the ratio of two-thirds to one, and that the expediting influence of bile in the presence of hydrochloric acid may be represented by the ratio of four to one. If, therefore, the bile be shut off from the intestinal canal, its expediting influence on the fat-splitting properties of pancreatic juice would not only be lost, but the retarding influence of the hydrochloric acid would be felt, and the work done by pancreatic juice before and after the shutting off of the bile would be represented by the ratio of four to two-thirds, or six to one. All physiologists agree that a certain amount of fat-splitting is necessary to fat-digestion, and we may, I think, take for granted that no unnecessary fat-splitting takes place during normal digestion. About 10 per cent. of fatty acid in oil gives the best spontaneous emulsion at body temperature (*a*), and more or less acid than this does not give a perfect

emulsion. We infer from this that about 10 per cent. of fatty acid is developed in intestinal fat preparatory to its emulsion and resorption. But whatever amount of fat-splitting normally occurs, we may be quite certain that natural selection has provided the conditions under which this amount of fat-splitting may take place. For example, the comparative immobility of the duodenum, its horseshoe shape, its diminution in calibre, and its close attachment to the head of the pancreas—all, no doubt, have an influence on the rate of passage of food-stuffs, and this rate, which is chiefly controlled by these and other anatomical conditions, was established to accord with normal digestive functions. By this mechanism the fats are exposed to the action of pancreatic juice just long enough to allow for whatever action that juice may have in fat-digestion. With these anatomical facts in mind, we are better prepared to appreciate the important part that bile plays in the intestinal digestion of fats. Let us suppose that under normal conditions the fats are exposed in the duodenum to the action of pancreatic juice for x minutes, which time is sufficient for whatever fat-splitting is necessary at this point. Now if the bile is cut off, the rate of passage of the food-stuffs, being controlled by anatomical conditions, would remain the same, and the fats would still be exposed to the action of the juice for only x minutes. But since in the absence of bile the pancreatic juice is able to accomplish only one-sixth of the fat-splitting which it normally does, it would follow that the fats would pass the duodenum with only one-sixth of the splitting that normally occurs; and since the splitting of fats is a necessary preliminary step in their digestion, it would follow that the fats would pass in great part undigested. This gives to bile a most important and definite position among the juices which assist in fat-digestion, since we have here pointed out one of the ways in which it exerts its well-known influence. We have now an explanation of the pale, fatty stool that occurs when the bile-ducts are occluded from any cause. The fatty stool will also occur when the pancreatic secretion is shut off from the intestine, but it should contain less fatty acid, and not have the paleness and putridity of the fatty stool due to occlusion of the bile-ducts. We should also have fatty stools when the intestinal juice is absent or deficient because of a failure in fat-resorption, and the characteristic of the fatty stool due to this cause would be the large amount of fatty acid it contained.

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III.—THE DIASTATIC ACTION OF PANCREATIC JUICE.

In recent years many observers have devoted much time and labor to the study of the action of unorganized ferments on food-stuffs. In this study amylopsin, the diastatic ferment of the pancreas, has been almost wholly neglected. In fact, as far as I am aware, no systematic study of the action of amylopsin on starch under the various conditions herein described has ever been made with pure pancreatic juice.[1]

Chittenden, Langley and Eves, and others have given us most valuable information on the diastatic action of saliva as modified by various conditions, suggested by the conditions under which diastatic ferments are supposed to act in the digestive tract; and in the absence of experimental work to determine the diastatic action of pancreatic juice, under such varying conditions, we have for the most part been forced to formulate our knowledge of the action of amylopsin from the experimental work done with ptyalin and other diastatic ferments. It may be that the diastatic action of the pancreatic juice will under all conditions be the same as that of saliva; yet, however this may be, there can be no doubt that it would be more desirable to have our knowledge of the diastatic action of pancreatic juice based upon experiments made with the juice itself rather than be forced to infer the extent of its action under diverse conditions from the action of other diastatic ferments under similar conditions.

In this statement I do not wish to appear to underrate the value of these studies or to insist that the principles of starch digestion, as obtained from the study of the diastatic action of saliva and malt, shall not be applied in explaining the diastatic action of pancreatic juice in the small intestine, but it goes with-

1 See the criticism of Hamburger, *Archiv. f. d. ges. Physiol.*, 1895, lx, p. 558.

out saying that confirmatory experiments made with pure pancreatic juice would substitute certainty for inference and place our knowledge of the intestinal digestion of starches on a much more satisfactory footing.

The work of pancreatic juice in carrying on the intestinal digestion of starchy foods is recognized as a digestive function second in importance to none. That experimenters have neglected this field of work is not therefore due to its unimportance, but rather to the difficulties in the way of obtaining pure pancreatic juice in quantities sufficient to carry on a systematic research. The purpose, therefore, of this paper is not only to record some experiments, which I recently have made, but also and more especially to call attention to a *method* of obtaining pure pancreatic juice from the rabbit in sufficient quantity for experimental purposes.

OPERATION FOR TEMPORARY PANCREATIC FISTULA IN THE RABBIT.

The operation for making a temporary pancreatic fistula in the rabbit was first described by me in the *Journal of Physiology*, 1891, xii, p. 80. The operation given here in detail is an improvement on the original, and makes it possible for any observer to obtain sufficient pancreatic juice for research. Experience has taught me that it is a waste of time to use any but the largest and strongest rabbits. With such animals the operation for temporary pancreatic fistula is easily and quickly done as follows. After removing the hair make an abdominal incision in the linea alba, on a level with the lower ribs, three centimetres long. Find the duodenum, which is easily reached high up in the right hypochondriac region lying close against the abdominal wall, and bring it through this opening. Run down the gut to a point where the peritoneum binds it so closely that it will not come through the opening without tearing the mesenteric attachment, and just at this point will be found the pancreatic duct as it passes through a leaf of the pancreas to enter the intestinal canal. Gently separate the mesenteric attachment of the gut so as to bring the latter through the abdominal opening without tearing blood-vessels and producing unnecessary hemorrhage. By holding the gut to the light the pancreatic duct can readily be observed and two points chosen, one on either side about two centimetres from

the papilla, for applying ligatures to the intestine. These points should be selected with an eye to the vascular distribution to the intestine, so that the blood-supply shall be disturbed as little as possible. The ligatures are tied at this point so as to occlude the lumen of the intestine; the ends are then passed around the body of the animal and tied in that position. If it be preferred small clamps, resembling prepuce clamps, may be used instead of ligatures. All of the intestines except that portion included between the clamps or ligatures is returned to the abdominal cavity *without section*. This arrangement will maintain the relative position of the parts, so that the portion of the mesentery holding the pancreatic duct cannot be disarranged by peristaltic contractions or by the subsequent movements of the animal. If a large abdominal wound is made it is sometimes necessary to close it partially by stitches, so that the intestine cannot be forced through the opening and in that way disarrange the parts. Care must be taken, however, that the abdominal wound be sufficiently open to permit the free passage of the mesentery carrying the pancreatic duct. The portion of the intestine included between the clamps or ligatures is now laid open opposite its mesenteric attachment and spread out on the abdominal wall. The lateral margins are packed with absorbent cotton to prevent bleeding. The pancreatic papilla can be observed on the exposed mucous membrane. In a short time it will open and pancreatic juice will exude. Into this papilla is inserted a small glass cannula to which has been attached an inch or two of rubber tubing. The cannula being in position, the exposed mucous membrane is covered with absorbent cotton, which may, if necessary, be saturated from time to time with warm physiological salt solution. The flow of juice may begin at once, or it may not commence for an hour after the insertion of the tube. When the pancreatic juice commences to flow it continues from five to eight hours. During this time the juice is collected by emptying the filled cannula from time to time and reinserting it. In this manner may be obtained from three to five c.c. of pancreatic juice uniform and powerful in physiological action. It is my custom to have two rabbits under operation at the same time, and in this way I never fail to obtain juice sufficient for an experiment. The pancreatic juice collected from both rabbits is mixed together in a single tube and afterward divided equally between the tubes containing the

digestive mixtures. In this way one is sure that all the tubes of a single experiment contain pancreatic juice uniform in physiological action.

INFLUENCE OF BILE AND HYDROCHLORIC ACID ON THE DIASTATIC ACTION OF PANCREATIC JUICE.

In the starch experiments here recorded the bile used was obtained from the same rabbits that furnished the pancreatic juice.

The method used in determining the rate of diastatic action was the one described by Pavy[1] under the title "Quantitative Determination of Sugar by the Ammoniated Cupric Test."

The time of each experiment was forty-five minutes. When the diastatic action was stopped by boiling the mixtures, the total volume of liquid in each tube was 60 c.c. One gram of wheat starch boiled in a definite quantity of water was used in each.

The hydrochloric acid used in these experiments was in the form of a 0.1 per cent. solution. I made a number of tests to determine the amount of this acid solution necessary to neutralize a given quantity of pancreatic juice and the results of these tests are noted elsewhere in this paper. Determinations were also made by Dr. W. H. Crane, who was given 5 minims of pure pancreatic juice and a sample of the 0.1 per cent. hydrochloric acid solution used in the experiments, with the request that he estimate the amount of the acid solution necessary to neutralize the 5 minims of juice.

Dr. Crane diluted the pancreatic juice with 5 c.c. of distilled water and added a drop of 0.5 per cent. alcoholic solution of dimethyl amido azo-benzol. Ten c.c. of 0.1 per cent. hydrochloric acid were diluted to 100 c.c., placed in a burette, and added drop by drop to the pancreatic juice. Neutralization was obtained with 4.5 c.c. of the 0.01 per cent. hydrochloric acid. Thus 5 minims of pancreatic juice required 0.45 c.c. of 0.1 per cent. hydrochloric acid for neutralization.

It may here also be noted that all the hydrochloric acid tubes, in the above or in subsequent experiments, contained from 2 to 10 c.c. of the hydrochloric acid solution in excess of the amount required to neutralize the pancreatic juice, and that the subsequent addition of bile or albumen was not sufficient to neutralize

1 Pavy, F. W.: "Physiology of the Carbohydrates," London, 1894.

this excess of free acid. All hydrochloric acid tubes therefore in the following experiments except those to which sodium carbonate was added contained free acid.

I submitted to Dr. Crane a fluid containing the following ingredients: pancreatic juice 10 minims, 0.1 per cent. hydrochloric acid solution 3 c.c., starch 1 gram, water 25 c.c., with the request that he determine whether or not the fluid contained free acid, and if acid how much bile would be required to neutralize the acid.

Dr. Crane found 27.4 c.c. of fluid in the test-tube. It was acid to dimethyl amido azo-benzol. Of the starchy fluid 13.7 c.c. were placed in a beaker and bile (0.9 c.c. of which had been diluted to 9.0 c.c. with water) introduced from a graduated pipette. Neutralization required 6.2 c.c. Thus 27.4 c.c. of the fluid are neutralized by 1.24 c.c. bile.

I would call attention to the fact that the mixture upon which the above report was made was taken as a sample of the hydrochloric acid tubes in the experiments on page 46. It must be noted, however, that this mixture contained the minimum amount of acid used in any of the digestive mixtures, and that this mixture was found to require 1.24 c.c. of bile to neutralize it; a much larger amount of bile than was used in any of the tubes, except tubes 1 and 2 of Experiment VII.

In these tubes, however, it will be noted that the 8 and 10 c. c. of the hydrochloric acid solution which these tubes respectively contained was sufficient to give to them an acid reaction.

Studying the following experiments, it is plain that bile slightly expedites the diastatic action of pancreatic juice. The expediting influence of the bile, however, is here shown to be so slight that the manner of its action scarcely merits discussion. It is important to observe that the bile does not exert an unfavorable influence on the diastatic action of pancreatic juice. But it is of much more importance to note that the 0.1 per cent. hydrochloric acid solution used in these experiments had only a slight retarding influence on the diastatic action of the ferment. A slight retardation is, however, seen in every case. Tube 3 in each of these experiments contains 3 c.c. of a 0.1 per cent. solution of hydrochloric acid, which gave to the contents a decided acid reaction. In experiments made to determine the amount of the hydrochloric acid solution necessary to neutralize pancreatic juice, it was

Experiment.	Pancreatic juice. Minims.	Water. c.c.	0.1 per cent. HCl. c.c.	Bile. Minims.	Starch. Gram.	Starch converted. Per cent.
I.						
1	10	60	0	4	1	30
2	10	57	3	4	1	27
3	10	57	3	0	1	23
4	10	60	0	0	1	30
II.						
1	10	60	0	8	1	50
2	10	57	3	8	1	50
3	10	57	3	0	1	37
4	10	60	0	0	1	40
III.						
1	10	60	0	6	1	32
2	10	57	3	6	1	32
3	10	57	3	0	1	20
4	10	60	0	0	1	25
IV.						
1	7	60	0	5	1	25
2	7	57	3	5	1	27
3	7	57	3	0	1	24
4	7	60	0	0	1	26
V.						
1	10	60	0	5	1	34
2	10	57	3	5	1	36
3	10	57	3	0	1	30
4	10	60	0	0	1	34
VI.						
1	10	60	0	8	1	34
2	10	57	3	8	1	36
3	10	57	3	0	1	24
4	10	60	0	0	1	28

found that 12 to 15 minims of the hydrochloric acid solution would destroy the alkalinity of 10 minims of pancreatic juice, and that 15 to 18 minims would give to it an acid reaction. Hence the amount of hydrochloric acid solution in these tubes was more than 2 c.c. in excess of the amount required to neutralize the alkalinity of the pancreatic juice, and yet in the presence of this excess of free hydrochloric acid the pancreatic juice had almost as much diastatic action as it had when acting alone in the presence of its own alkaline salts.

Of yet greater interest are the tubes marked 2 in the above experiments. Each of these tubes contained not only 3 c.c. of the hydrochloric acid solution, but also a certain amount of bile. A study of these tubes shows us that the bile not only neutralized the slight retarding influence of the free hydrochloric acid, but

also furnished the conditions in the presence of this acid for pancreatic juice to do its most rapid diastatic work. The tubes containing both bile and pancreatic juice, in every experiment except one, lead in the amount of diastatic work done. In the experiment in which the bile and hydrochloric acid tube fails to do the most work, the failure is probably due to the small amount of bile used. Whatever may be the explanation, it is an important physiological fact that bile, when added to pancreatic juice acting in the presence of a small quantity of free hydrochloric acid, will not only neutralize the retarding influence which the free acid has on the diastatic action of pancreatic juice, but will in doing so furnish the most favorable conditions for the action of this ferment. And it is also important to note that bile will accomplish this result without neutralizing the acid completely, thus showing that the favorable influence of the bile on the diastatic action of pancreatic juice is not simply one of acid neutralization.

The following experiment shows even more clearly the value of bile when pancreatic juice is acting in the presence of free

EXPERIMENT VII.

	Pancreatic juice. Minims.	Water. c.c.	0.1 per cent. HCl. c.c.	Bile. Minims.	Starch. Gram.	Starch reduced. Per cent.
1	12	52	8	20	1	40
2	12	48	12	30	1	24
3	12	48	12	0	1	12
4	12	52	8	0	1	14
5	12	60	0	0	1	30

hydrochloric acid. Here the retarding influence of 8 and 12 c.c. of a 0.1 per cent. hydrochloric acid solution is very marked, but in tube 1, containing 8 c.c. of the hydrochloric acid solution, 20 minims of bile not only neutralizes the retarding action of the hydrochloric acid, but also enables the pancreatic juice to do even more diastatic work than is done in tube 5, where the pancreatic juice is acting apart from the influence of either hydrochloric acid or bile. In tube 2, 30 minims of bile enables 12 minims of pancreatic juice acting in the presence of 12 c.c. of hydrochloric acid solution to digest 24 per cent. of starch instead of 12 per cent., the amount digested in tube 3, in which the same quantity of pancreatic juice acted in the presence of 12 c.c. of the hydrochloric acid solution without the assistance of the bile.

In Experiment VIII, even more graphically than in any that has preceded it, is shown the retarding influence of free hydrochloric acid on the diastatic action of pancreatic juice and the value of bile in neutralizing this retarding action. Here 12 c.c. of the hydrochloric acid solution almost destroys the diastatic action of 15 minims of pancreatic juice, and 8 c.c. of the acid solution greatly retards its action, while 7 minims of bile is sufficient to neutralize entirely the retarding influence of 8 c.c. of the acid solution.

EXPERIMENT VIII.

	Pancreatic juice. Minims.	Water. c.c.	0.1 per cent. HCl. c.c.	Bile. Minims.	Starch. Gram.	Starch reduced. Per cent.
1	15	60	0	0	1	25
2	15	52	8	0	1	14
3	15	48	12	0	1	2
4	15	52	8	7	1	26
5	15	48	12	7	1	8
6	15	52	8	0	1	18
7	15	48	12	0	1	5
8	15	52	8	7	1	25
9	15	48	12	7	1	6

The preceding experiments clearly establish the fact that bile is not necessary to the diastatic action of pancreatic juice acting alone uninfluenced by hydrochloric acid, but that bile may be of the greatest assistance to pancreatic juice, indeed almost necessary to its full diastatic action, when the former is acting in the presence of free hydrochloric acid, and that bile can serve this purpose without neutralizing all of the free acid present.

INFLUENCE OF ACID ALBUMEN ON THE DIASTATIC ACTION OF PANCREATIC JUICE.

In Experiment IX, the egg albumen was mixed with the hydrochloric acid solution before the pancreatic juice was added to the tube, and here it will be seen that 0.3 gram of egg albumen exercised very much the same influence on pancreatic juice acting in the presence of free hydrochloric acid that the bile did in the previous experiments. Tubes 2, 4 and 6 demonstrate that acid albumen very materially expedites the diastatic action of pancreatic juice. Tubes 6, 7 and 8 of this experiment contain each 10 minims of bile. In tube 6 the presence of the bile seems to add little to the diastatic action of the pancreatic juice, since almost

the same percentage of starch is digested in tubes 2 and 4, which do not contain bile, but in other respects resemble tube 6. In tube 7, however, we learn by comparison with tubes 3 and 5 that the 10 minims of bile had a very decided influence in increasing the diastatic action of the pancreatic juice in the conditions under which it is acting. In tube 7 of this experiment 0.3 gram of egg

EXPERIMENT IX.

	Pancreatic juice. Minims.	Water. c.c.	0.1 per cent. HCl. c.c.	Egg Albumen. Gram.	Bile. Minims.	Starch. Gram.	Starch reduced. Per cent.
1.....	10	60	0	0	0	1	23
2.....	10	56	4	0.3	0	1	31
3.....	10	52	8	0.3	0	1	19
4.....	10	56	4	0.3	0	1	37
5.....	10	52	8	0.3	0	1	15
6.....	10	56	4	0.3	10	1	38
7.....	10	52	8	0.3	10	1	25
8.....	10	60	0	0	10	1	25

albumen was not sufficient to neutralize fully the retarding action of 8 c.c. of the hydrochloric acid solution; the addition of 10 minims of bile, however, accomplished this. The failure of bile to increase further the diastatic action of pancreatic juice in tube 6 is due to the fact that the 0.3 gram of egg albumen is almost if not quite sufficient to neutralize the 4 c.c. of hydrochloric acid solution, and for this reason the further addition of bile or egg albumen would not materially increase the action of the pancreatic juice.

INFLUENCE OF SODIUM CARBONATE ON THE DIASTATIC ACTION OF PANCREATIC JUICE.

Experiment X was planned for the purpose of studying the influence of sodium carbonate on the diastatic action of pancreatic juice acting under various conditions. A 1 per cent. solution of sodium carbonate was used. Tubes 2 and 3 of this experiment show that 2 and 5 c.c. of the sodium carbonate solution almost entirely destroy the diastatic action of 5 minims of pancreatic juice; while tubes 4 and 5 show that 5 minims of bile have a decided influence in neutralizing the retarding influence which the soda solution has upon the diastatic action of pancreatic juice. Tubes 6 and 7 indicate that the 0.6 gram of egg albumen also had some influence in neutralizing the retarding action of the soda.

EXPERIMENT X.

	Pancreatic juice. Minims.	Water. c.c.	1 per cent. Na_2CO_3. c.c.	Bile. Minims.	Egg Albumen. Gram.	0.1 per cent. HCl. c.c.	Starch. Gram.	Starch reduced. Per cent.
1	5	60	0	0	0	0	1	50
2	5	58	2	0	0	0	1	7
3	5	55	5	0	0	0	1	2
4	5	58	2	5	0	0	1	15
5	5	55	5	5	0	0	1	6
6	5	58	2	0	0.6	0	1	10
7	5	55	5	0	0.6	0	1	4
8	5	52	5	0	0.6	3	1	13

If hydrochloric acid be added to a mixture in which pancreatic juice is acting on starch in the presence of sodium carbonate the acid will neutralize the retarding influence of the alkali. This is shown in tube 8. This action may be simply one of chemical neutralization, but whatever the explanation, the observation is interesting as showing that the diastatic power of pancreatic juice in a strongly alkaline mixture may be increased by the addition of a small quantity of hydrochloric acid.

EXPERIMENT XI.

	Pancreatic juice. Minims.	Water. c.c.	0.1 per cent. Na_2CO_3. c.c.	Bile. Minims.	Starch. Gram.	Starch reduced. Per cent.
1	4	60	0	0	1	50
2	4	58	2	0	1	5
3	4	55	5	0	1	3
4	4	58	2	4	1	11
5	4	55	5	4	1	13
6	....	60	0	4	1	8
7	....	60	0	4	1	8

Experiment XI again demonstrates the destructive action of sodium carbonate on the diastatic action of pancreatic juice, and also shows the value of bile in neutralizing this retarding influence. In tubes 6 and 7 it is shown that bile itself has some diastatic power.

CONCLUSIONS.

1. A small quantity of free hydrochloric acid has little or no retarding influence on the diastatic action of pancreatic juice.

2. Larger quantities of free hydrochloric acid very materially retard the diastatic action of pancreatic juice; in one experiment 12 c.c. of a 0.1 per cent. solution of hydrochloric acid almost de-

stroyed the diastatic action of 15 minims of pancreatic juice acting in a mixture of 60 cubic centimetres volume; in another experiment the same quantity of acid reduced by two-thirds the diastatic action of 12 minims of pancreatic juice. The facts that different specimens of pancreatic juice vary in their degree of alkalinity and in their diastatic power make it impossible to formulate precise statements concerning the influence which definite quantities of acid will have on definite quantities of pancreatic juice.

3. Acid proteids in small quantities slightly increase the diastatic action of pancreatic juice. Neutral proteids, therefore, when added to pancreatic juice acting in the presence of free hydrochloric acid will not only neutralize the retarding action of the acid on the diastatic action of the juice, but they will also by the formation of acid albumen assist materially the pancreatic juice in its work.

4. Sodium carbonate has a very destructive influence on the diastatic action of pancreatic juice. Two cubic centimetres of a 1 per cent. solution of sodium carbonate almost totally destroys the diastatic action of 5 minims of pancreatic juice acting in a mixture of 60 cubic centimetres volume.

5. Bile has no retarding influence on the diastatic action of pancreatic juice; in fact, it slightly expedites its action.

6. Bile not only neutralizes the retarding influence which free hydrochloric acid has upon the diastatic action of pancreatic juice, but in the presence of free hydrochloric acid it very materially expedites the action of the juice. Here again it is impossible to formulate rules as to the exact amount of bile necessary to neutralize the retarding influence of a definite quantity of hydrochloric acid, and thus give to a definite quantity of pancreatic juice its greatest diastatic power. In the above experiments, however, it will be seen that four to eight minims of bile were sufficient to neutralize the retarding influence of from four to eight cubic centimetres of a 0.1 per cent. hydrochloric acid solution without destroying the acid reaction of the mixture and thus to give to pancreatic juice its greatest diastatic power.

7. Bile has a marked influence in diminishing the retarding influence which sodium carbonate has upon the diastatic action of pancreatic juice.

8. Bile itself has some diastatic power.

Not the least interesting point in these conclusions is the suggestion that bile may play a not unimportant part in the intestinal digestion of starches. If there be any free acid in the food, as it is discharged from the stomach into the duodenum, the bile will neutralize this acid and thereby assist the acid proteïds discharged with the starches through the pylorus, in furnishing the most favorable conditions for the diastatic action of pancreatic juice. And possibly of even more importance is the fact that bile will limit the destructive action of sodium carbonate on the diastatic action of amylopsin.

In conclusion, I wish to express my thanks to Dr. Frank Southgate, who has assisted me very greatly in the arduous details of these experiments.

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IV.—INFLUENCE OF BILE, OF ACIDS, AND OF ALKALIES ON THE PROTEOLYTIC ACTION OF PANCREATIC JUICE.

Dr. Southgate and I published, in 1895, the results of some experiments on the influence of bile and combined hydrochloric acid upon the proteolytic action of pancreatic juice.[1] These results have been questioned, by Chittenden and Albro,[2] on the ground that similar results are not obtained when pancreatic extracts are used in the place of pancreatic juice. It is perhaps hardly necessary to point out that experiments with pancreatic extracts cannot be taken as decisive as to what will happen when pancreatic juice is employed. Nevertheless, in view of the position taken by Chittenden and Albro, I have, with the aid of Dr. Southgate, made further experiments on the subject.

METHOD.

All the pancreatic juice, employed in these experiments, was obtained from rabbits by a method devised by me and first published in this journal, xii, p. 72, 1891, and recently improved and published in detail in the *American Journal of Physiology*, ii, p. 483, 1899.

The bile, used in all of these experiments, was fresh rabbits' bile taken from the same animals which furnished the pancreatic juice, and in nearly all of the experiments it was filtered before using.

The proteid employed was purified and dried blood fibrin prepared by washing with water, extracting with cold and boiling alcohol, and lastly with ether. It was then ground to a fine powder, dried and weighed at 100° C. All of the weighings were made by a competent assistant and the powders were de-

1 *Medical Record*, 1895, p. 878.

2 *American Journal of Physiology*, i, p. 307, 1898.

livered to me along with carefully weighed and marked filter papers. At the close of each experiment the filter papers, containing the undigested residues of fibrin, were dried and weighed at 100° C. and the result sent to me. The assistant who did the weighing knew nothing of the contents of the digestion tubes or returned filter papers. This precaution was taken for the purpose of avoiding such slight errors as might unconsciously occur from a knowledge of the contents of the weighings. The digestive mixtures, used in these experiments, were placed in test-tubes of large calibre, especially prepared for this purpose, which were kept in a water bath at 38° C. They were equally stirred while the digestive process was going on. At the close of the experiment the undigested residues were collected on weighed and marked filters, and after being thoroughly washed were delivered to the assistant to be dried and weighed as previously described.

The experiments recorded in this paper were, as a rule, commenced by the collection of the pancreatic juice about eight o'clock in the morning, so that by three o'clock in the afternoon from three to four c.c. of pancreatic juice would be obtained. During this time the pancreatic juice, from the two or three rabbits operated upon, was placed in a common receptacle and kept in a cool place. The juice was equally divided between the digestion tubes of one or more experiments. This insured the relative accuracy of the results, since each digestion mixture of an experiment contained the same quantity of pancreatic juice uniform in physiological action. Corresponding tubes of different experiments, however, cannot be compared because of the variation in strength of the different specimens of pancreatic juice.

The digestion experiments were commenced about four o'clock in the afternoon and continued until about eleven o'clock at night. The filter papers containing the undigested residues of fibrin were placed in the oven about midnight; in some experiments earlier, in others later. It will thus be seen that an experiment required from seventeen to eighteen hours of constant watching.

I am not prepared to say that experiments made with pancreatic juice, collected under the above conditions, are more reliable than those made with pancreatic extracts. Yet I fail to see why results obtained from natural pancreatic juice do not constitute a method more nearly resembling the conditions under which proteolysis takes place in the duodenum; and further than

this it occurs to me that if the fresh bile and pancreatic juice of the same animal be commingled in the digestion of fibrin the conditions would even more closely resemble pancreatic proteolysis as it occurs in the carnivora.

In some comparative anatomy studies,[1] made in 1891, I called attention to the fact that the more exclusively carnivorous the animal the more certainly will the bile and pancreatic juice be poured into the duodenum through a common opening, and the more closely will this opening approach the pylorus. In the lion this opening is only 6 cm. from the pylorus; in the tiger 5 cm.; in the leopard 4 cm.; in the wild cat 3 cm.; and in a number of other carnivora this distance is less than 2 cm. In a few animals such as the bear, the badger, and the fox, there is an apparent exception to the above rule. In these animals, however, although the ducts do not have a common opening, they enter the duodenum almost upon the same level, so that, by this arrangement, the bile and pancreatic juice are mixed directly they are poured into the intestines.

That this preliminary mixing of bile and pancreatic juice so near the pylorus in carnivorous animals serves a physiological purpose cannot be doubted, and it seems very improbable that this purpose is served solely in connection with fat digestion[2] and without reference to the far more important proteid digestion in these animals.

However this may be, this arrangement suggests that the ideal method of studying pancreatic proteolysis as it takes place in the carnivora, is one that uses the pure juice and the fresh bile of the same animal, and subjects them to preliminary mixing before they are brought into contact with proteids. This suggestion has even more importance when one remembers, as Chittenden and Albro have so clearly noted, "how radically bile from different species of animals differs in composition."

When rabbit's bile is used with rabbit's pancreatic juice, we are imitating conditions which nature has evolved for the digestion of food-stuffs in the small intestine of this animal. It would seem, therefore, that results obtained by this method would be of value in assisting to interpret the digestive processes normally going on in the intestine of man. Surely such experiments can-

1 *Medicine*, December, 1895.

2 This journal, xii, p. 72, 1891.

not be less valuable than those obtained from pancreatic extracts commingled with the bile of different species of animals.

TABLE A.—*Influence of Bile on the Proteolytic Action of Pancreatic Juice.*

Number of experiment.	Duration in hours.	Fibrin in grams.	Water in c.c.	Bile in c.c.	Pancreatic juice in c.c.	Loss of weight in grams.
I	8	.398	10	0	.246	.061
	8	.399	10	.369	.246	.093
	8	.399	10	.369	.246	.089
II	7	.400	6	0	.246	.075
	7	.400	6	.184	.246	.105
	7	.400	6	.184	.246	.096
III	6	.400	7	0	.246	.065
	6	.400	7	.184	.246	.097
	6	.400	7	.184	.246	.089
IV	8	.400	10	0	.369	.078
	8	.400	10	.246	.369	.091
	8	.404	10	.246	.369	.109
V	6	.400	7	0	.431	.146
	6	.400	7	.184	.431	.158
	6	.400	7	.184	.431	.179
VI	8	.400	6	0	.492	.097
	8	.400	6	.184	.492	.130
	8	.400	6	.184	.492	.127
VII	6	.400	6	0	.616	.146
	6	.400	6	.184	.616	.187
VIII	4	.400	7	0	.616	.096
	4	.400	7	.184	.616	.122
IX	6	.400	6	0	.616	.113
	6	.400	6	.184	.616	.130
X	8	.407	10	0	.677	.070
	8	.402	10	.369	.677	.087
	8	.407	10	.369	.677	.079
XI	8	.402	10	0	.739	.098
	8	.401	10	.369	.739	.134
	8	.401	10	.369	.739	.123
XII	6	.400	6	0	.739	.130
	6	.400	6	.184	.739	.178

INFLUENCE OF BILE ON THE PROTEOLYTIC ACTION OF PANCREATIC JUICE.

Chittenden and Albro conclude, "That the addition of fresh bile to a neutral pancreatic extract does not give rise to any great

degree of stimulation, *i.e.*, the proteid digesting power of the enzyme is not remarkably increased." And they further add that, "in no one of our experiments do we find a confirmation of the results reported by Rachford and Southgate, who found on an average that the proteolytic action of pancreatic juice was increased one-fourth by the addition of bile."

As the preceding table will show, I, on the other hand, am unable, working with pancreatic juice, to confirm the results, as to the impotency of bile, which Chittenden and Albro obtained working with pancreatic extracts.

An examination of this table clearly demonstrates that when fresh rabbits' bile is added to fresh rabbits' pancreatic juice, acting upon neutral fibrin, the proteolytic power of the juice will be markedly stimulated, and in most of the experiments the juice is able to do one-fourth more work by reason of the presence of the bile.

INFLUENCE OF BILE ON PROTEOLYSIS CARRIED ON BY ORGANIZED FERMENTS.

In the following table I have grouped a series of experiments in which the digestive process was allowed to go on for twelve or more hours. The putrid odor, which developed in all of these tubes, showed that organized ferments, along with the trypsin, were taking part in the proteolysis. While these experiments have no value in the study of pancreatic proteolysis, yet when the results obtained from this series are compared with those

TABLE B.—*Influence of Bile on Proteolysis Carried on by Organized Ferments.*

Number of experiment.	Duration in hours.	Fibrin in grams.	Water in c.c.	Bile in c.c.	Pancreatic juice in c.c.	Loss of weight in grams.
I	14	.400	10	0	.369	.172
	14	.400	10	.554	.369	.151
II	14	.397	10	0	.616	.118
	14	.400	10	.369	.616	.091
	14	.406	10	.369	.616	.096
III	12	.398	10	0	.862	.129
	12	.400	10	.862	.862	.125
	12	.399	10	.862	.862	.129
IV	12	.389	10	0	.924	.139
	12	.399	10	.369	.924	.146

obtained from a study of Table A, one is led to conclude that bile has an influence in limiting or retarding the fermentation which is carried on by organized ferments. In only one of these experiments, No. IV, do we find the bile tube doing more proteolytic work than the tube in which the pancreatic juice acted without the influence of bile. In Experiment III the bile tubes and the non-bile tube do practically the same work, while in Experiments I and II, which lasted fourteen hours, we find that the pancreatic juice acting alone does more proteolytic work than it does when acting in the presence of bile. The inference seems plain that in the fourteen-hour experiments the bile was able to so limit bacterial action that the pancreatic tubes containing no bile were able to do the most proteolytic work. I am therefore of the opinion that rabbits' bile, while it expedites the fermentation carried on by the pancreatic enzyme, trypsin retards the albuminous fermentations carried on by organized ferments. This view seems to favor the opinion that bile by limiting bacterial action may act as an intestinal antiseptic.

INFLUENCE OF COMBINED HYDROCHLORIC ACID UPON THE PROTEOLYTIC ACTION OF PANCREATIC JUICE.

In the following experiments the degree of saturation of the fibrin was estimated from careful experiments, made expressly to detemine the point of saturation of the fibrin used in these experiments, with the carefully tested hydrochloric acid solution here employed. The presence or absence of free hydrochloric acid was determined by a 0.5 per cent. alcoholic solution of dimethyl-amido azo-benzol.

Chittenden and Albro state that "the combined or free acid which passes from the stomach through the pylorus is without doubt quickly removed by absorption or destroyed by neutralization." And that, "the evidence is certainly in favor of the view that the contents of the duodenum are generally alkaline." Apart from this statement of Chittenden and Albro I have not been able to find any evidence pointing to alkalinity of the contents of the duodenum in the carnivora. At what part of the intestinal canal in carnivorous animals its contents cease to be acid and take on an alkaline reaction does not appear in their paper, nor is this matter made clear by the research of Moore and Rockwood,[1]

1 This journal, xxi, p. 373, 1897.

which they quote, as offering the best evidence of the early disappearance of acid in the upper portion of the small intestine. All the evidence, in fact, which these authors present leads to the inference that in carnivorous animals fed on proteids the "upper portion" of the small intestine is usually acid and the "lower portion" is faintly alkaline. These results they obtained from the dog and cat, the only carnivora upon which they operated. And I may add that these views are in accord with those generally accepted by physiologists to-day.

The experiments recorded in the tables which follow were planned with the purpose of studying the influence which these conditions would have on the proteolytic action of pancreatic juice. In this table the "degree of saturation," refers to the condition of the fibrin before the pancreatic juice was added. In none of the tubes, except one of Experiment XVI, was there any free acid present.

By a study of Experiments I to XII inclusive, we are led to believe that fibrin one-half saturated with hydrochloric acid, is as readily acted upon by pancreatic juice as is neutral fibrin. The amount of proteolysis that occurred in the tubes containing hydrochloric acid will be found almost, if not quite, to equal that which took place in the tubes where pancreatic juice was acting upon neutral fibrin.

If, however, we study Experiments XIII, XIV, XV, XVI and XVII, we find that when the fibrin was nine-tenths saturated with hydrochloric acid the proteolytic power of pancreatic juice was considerably retarded. In Experiment XVI we also note the more marked influence which free acid had in retarding the proteolytic action of pancreatic juice.

The results of these experiments differ radically from those of Chittenden and Albro, who found "that even a few thousands of 1 per cent. of combined hydrochloric acid sufficed to exert an inhibitory influence on proteolysis, and with a sufficient amount of combined acid alone proteolysis may be almost completely checked." My own experiments, however, lead to the conclusion that when rabbits' pancreatic juice is added to fibrin, one-half saturated with hydrochloric acid, it will do almost as much work in proteolysis as when added to neutral fibrin. When, however, the fibrin is nine-tenths saturated with hydrochloric acid the proteolytic action of the juice is retarded. And Experiment No.

XVI indicates that a certain amount of pancreatic proteolysis may go on even in the presence of free hydrochloric acid.

TABLE C.—*Influence of Combined HCl on the Proteolytic Action of Pancreatic Juice.*

Number of experiment.	Duration in hours.	Fibrin in grams.	Degree of saturation.	Water in c.c.	Pancreatic juice in c.c.	Loss of weight in grams.
I	4½	.398	0	7	.246	.114
	4½	.400	½	7	.246	.105
II	8	.400	0	7	.246	.065
	8	.400	½	7	.246	.073
III	7	.400	0	6	.246	.065
	7	.400	½	6	.246	.073
IV	7	.400	0	7	.309	.217
	7	.400	½	7	.309	.225
V	6½	.400	0	7	.369	.146
	6½	.400	½	7	.369	.162
VI	6	.400	0	7	.431	.130
	6	.400	½	7	.431	.179
VII	8	.400	0	6	.492	.121
	8	.400	½	6	.492	.121
VIII	8	.400	0	6	.554	.097
	8	.400	½	6	.554	.031
IX	6	.400	0	6	.616	.113
	6	.400	½	6	.616	.113
X	6	.400	0	6	.616	.146
	6	.400	½	6	.616	.105
XI	4	.400	0	7	.616	.096
	4	.400	½	7	.616	.113
XII	6	.400	0	6	.739	.130
	6	.400	½	6	.739	.162
XIII	8	.398	0	10	.246	.161
	8	.400	9/10	10	.246	.057
XIV	8	.389	0	10	.246	.074
	8	.401	9/10	10	.246	.045
XV	8	.400	0	10	.369	.047
	8	.399	9/10	10	.369	.055
XVI	14	.400	0	10	.369	.172
	14	.400	9/10	10	.369	.157
	14	.400	9/10	10	.369	.162
	14	.400	free acid	10	.369	.191
XVII	12	.384	0	10	.924	.139
	12	.401	9/10	10	.924	.111

INFLUENCE OF BILE AND COMBINED HYDROCHLORIC ACID ON THE PROTEOLYTIC ACTION OF PANCREATIC JUICE.

In these experiments "the degree of saturation" refers to the condition of the fibrin before the bile and pancreatic juice are brought into contact with it. That is to say, I have attempted to study the influence which definite quantities of bile and pancreatic juice would have upon fibrin one-third, one-half, seven-tenths, and nine-tenths, saturated with hydrochloric acid. In none of the digestion tubes of this table was there any free acid.

In studying the following table one notes that in experiments from I to V inclusive 0.5 grams of fibrin, one-third saturated with hydrochloric acid, were delivered to definite quantities of bile and pancreatic juice, and that in every instance slightly more work was done in the tubes containing acid fibrin than in those in which neutral fibrin was acted upon by pure pancreatic juice apart from the influence of bile and combined hydrochloric acid.

On further examination of this table one is struck with the fact that in the twelve experiments from VI to XVII inclusive, 0.4 grams of fibrin, one-half saturated with hydrochloric acid, were delivered to definite quantities of bile and pancreatic juice, and that here again the greatest amount of proteolysis, in all except three, took place in the tube in which the bile and pancreatic juice were acting upon acid proteids.

Again, referring to the table, one finds that the four experiments, from XVII to XXI, show a slight decrease in proteolysis in the tubes containing fibrin seven-tenths saturated with hydrochloric acid.

In the last seven experiments embraced in the above table, an effort was made to study the influence of definite quantities of bile and pancreatic juice acting upon fibrin nine-tenths saturated. Of these seven experiments the combined hydrochloric acid retarded the proteolytic action of the pancreatic juice in all but one. Yet the retardation in most of these experiments was not very great.

A careful examination of this table does not enable me to agree with Chittenden and Albro, that "combined acid alone tends to retard pancreatic proteolysis, and the addition of bile to such mixtures increases still further the extent of retardation." On the other hand, I am led to the conclusions that a small quantity of combined hydrochloric acid, not greater than one-half

TABLE D.—*Influence of Bile and Combined Hydrochloric Acid on the Proteolytic Action of Pancreatic Juice.*

Number of experiment.	Duration in hours.	Fibrin in grams.	Degree of saturation with HCl.	Water in c.c.	Bile in c.c.	Pancreatic juice in c.c.	Loss of weight in grams.
I	4½	.501	0	20	0	.246	.054
	4½	.502	⅛	20	.616	.246	.068
	4½	.500	⅛	20	.616	.246	.069
II	4½	.502	0	20	0	.309	.076
	4½	.501	⅛	20	.616	.309	.088
	4½	.502	⅛	20	.616	.309	.095
III	4	.500	0	6	0	.369	.082
	4	.500	⅛	6	.123	.369	.086
IV	4¾	.502	0	20	0	.492	.083
	4¾	.500	⅛	20	616	.492	.078
V	4¾	.500	0	7	0	.677	.092
	4¾	.500	⅛	7	369	.677	.104
	4¾	.500	⅛	7	.369	.677	.094
	4¾	.500	⅛	7	369	.677	.092
VI	7	.400	0	6	0	.246	.065
	7	.400	⅛	6	.184	.246	.130
VII	8	.400	0	7	0	.246	.065
	8	.400	⅛	7	.184	.246	.130
VIII	7	.400	0	7	0	.309	.217
	7	.400	⅛	7	.184	.309	.240
IX	6½	.400	0	7	0	.369	.146
	6½	.400	⅛	7	.184	.369	.146
X	6	.400	0	7	0	.431	.130
	6	.400	⅛	7	.184	.431	.195
XI	4½	.400	0	7	0	.431	.114
	4½	.400	⅛	7	.184	.431	.127
XII	8	.400	0	6	0	.492	.121
	8	.400	⅛	6	.184	.492	.097
XIII	8	.400	0	6	0	.554	.097
	8	.400	⅛	6	.184	.554	.178
XIV	4	.400	0	7	0	.616	.096
	4	.400	⅛	7	.184	.616	.066
XV	6	.400	0	6	0	.616	.113
	6	.400	⅛	6	.184	.616	.146

TABLE D.—(*Continued.*)

Number of experiment.	Duration in hours.	Fibrin in grams.	Degree of saturation with HCl.	Water in c.c.	Bile in c.c.	Pancreatic juice in c.c.	Loss of weight in grams.
XVI.....	6	.400	0	6	0	.616	.146
	6	.400	$\frac{1}{2}$	6	.184	.616	.195
XVII	6	.400	0	6	0	.739	.130
	6	.400	$\frac{1}{2}$	6	.184	.739	.194
XVIII....	$4\frac{1}{2}$	.500	0	20	0	.246	.054
	$4\frac{1}{2}$	.500	$\frac{7}{10}$	20	.616	.246	.058
	$4\frac{1}{2}$	.500	$\frac{7}{10}$	20	.616	.246	.050
XIX.....	$4\frac{1}{2}$	.500	0	20	0	.309	.076
	$4\frac{1}{2}$	.500	$\frac{7}{10}$	20	.616	.309	.061
	$4\frac{1}{2}$	.500	$\frac{7}{10}$	20	.616	.309	.067
XX	4	.500	0	20	0	.369	.082
	4	.500	$\frac{7}{10}$	20	.616	.369	.081
	4	.500	$\frac{7}{10}$	20	.616	.369	.078
XXI.....	$4\frac{3}{4}$	.500	0	7	0	.677	.092
	$4\frac{3}{4}$	.500	$\frac{7}{10}$	7	.739	.677	.077
	$4\frac{3}{4}$	.500	$\frac{7}{10}$	7	.739	.677	.078
	$4\frac{3}{4}$	.500	$\frac{7}{10}$	7	.739	.677	.076
XXII.....	14	.400	0	10	0	.309	.138
	14	.400	$\frac{9}{10}$	10	.369	.309	.101
	14	.400	$\frac{9}{10}$	10	.369	.309	.102
XXIII...	14	.400	0	10	0	.369	.172
	14	.400	$\frac{9}{10}$	10	.554	.369	.126
	14	.400	$\frac{9}{10}$	10	.554	.369	.119
XXIV....	8	.400	0	10	0	.369	.078
	8	.400	$\frac{9}{10}$	10	.246	.369	.088
	8	.400	$\frac{9}{10}$	10	.246	.369	.088
XXV.....	14	.400	0	10	0	.616	.118
	14	.400	$\frac{9}{10}$	10	.369	.616	.051
	14	.400	$\frac{9}{10}$	10	.369	.616	.056
XXVI....	8	.400	0	10	0	.677	.070
	8	.400	$\frac{9}{10}$	10	.569	.677	.050
	8	.400	$\frac{9}{10}$	10	.569	.677	.056
XXVII..	12	.400	0	10	0	.862	.129
	12	.400	$\frac{9}{10}$	10	.862	.862	.114
	12	.400	$\frac{9}{10}$	10	.862	.862	.100
XXVIII	12	.400	0	10	0	.924	.139
	12	.400	$\frac{9}{10}$	10	.369	.924	.090

saturation, will but slightly diminish the proteolytic action of rabbits' pancreatic juice, and that when rabbits' bile is added to these mixtures an increase in proteolysis is attained.

My experiments indicate, however, that when hydrochloric acid is present in such quantities as to more than half saturate the fibrin it somewhat retards the proteolytic action of pancreatic juice, even though bile be present, and still greater retardation occurs with increasing quantities of hydrochloric acid. Nine-tenths' saturation considerably retards but by no means destroys the proteolytic action of pancreatic juice.

INFLUENCE OF BILE AND FREE HYDROCHLORIC ACID ON THE PROTEOLYTIC ACTION OF PANCREATIC JUICE.

The study of this question is attempted in the following table. In all the experiments included in this table the acid tubes contained free hydrochloric acid, even after the bile and pancreatic juice were added, and a number of these tubes, as will be seen, contained acid far in excess of the amount required to neutralize the bile and pancreatic juice and saturate the fibrin. The acid determinations were made with 0.5 per cent. alcoholic solution of dimethyl-amido azo-benzol.

TABLE E.—*Influence of Bile and Free Hydrochloric Acid on the Proteolytic Action of Pancreatic Juice.*

Number of experiment.	Duration in hours.	Fibrin in grams.	HCl. in grams.	Water in c.c.	Bile in c.c.	Pancreatic juice in c.c.	Reaction.	Loss of weight in grams.
I	8	.502	0	10	....	.184	....	.083
	8	.499	.007	10	....	.184	Acid.	.019
II	14	.100	0	10	0	.309	....	.108
	14	.400	.007	10	.369	.309	Acid.	.085
	14	.400	.007	10	.369	.309	Acid.	.084
	14	.400	.010	10	.369	.309	Acid.	.080
	14	.400	.014	10	.369	.309	Acid.	.036
III	4¾	.400	0	20	0	.309	....	.098
	4¾	.400	.007	20	.616	.309	Acid.	.0.9
	4¾	.400	.007	20	.616	.309	Acid.	.049
IV	4½	.500	0	20	0	.309	....	.076
	4½	.500	.007	20	.616	.309	Acid.	.041
	4½	.500	.007	20	.616	.309	Acid.	.037
	4½	.500	.007	20	.616	.309	Acid.	.047
V	14	.400	0	10	0	.369	....	.172
	14	.400	.007	10	0	.369	Acid.	.091

In every acid tube of the five experiments here recorded a marked retardation in proteolysis occurred, but in none of them was the proteolytic power of the pancreatic juice wholly destroyed. In most of the tubes the amount of work done by the bile and pancreatic juice, acting upon fibrin supersaturated with hydrochloric acid, was almost half as great as that done by pure pancreatic juice acting upon neutral fibrin. Under the conditions, therefore, noted in the above experiments free hydrochloric acid will greatly inhibit, but will not wholly destroy, the proteolytic action of rabbits' pancreatic juice, acting in the presence of rabbits' bile upon blood fibrin.

INFLUENCE OF SODIUM CARBONATE UPON THE PROTEOLYTIC ACTION OF DILUTE SOLUTIONS OF PANCREATIC JUICE.

By reference to the preceding tables it can readily be seen that the proteolytic action of pancreatic juice is greatly weakened by dilution. That is to say, the most proteolytic work, as a rule, was done in those tubes which contained the largest quantities of pancreatic juice and the smallest quantities of water, and the least amount of proteolytic work was done in those tubes which contained the smallest amounts of juice and the largest amounts of water.

Upon theoretical grounds this would seem to be a serious drawback to thorough proteolysis in the intestinal canal, since this process requires hours for its completion, and occurs not alone in the duodenum, but probably throughout the greater portion of the small intestine.

These inferences and deductions naturally suggest an inquiry into the influence which the sodium carbonate and alkaline intestinal contents of the lower ileum and jejunum would have upon the proteolytic action of pancreatic juice. The few experiments in the following table were designed to study this question.

The favorable influence which sodium carbonate has on the proteolytic action of dilute solutions of pancreatic juice is here shown. This influence is most marked in Experiment II, wherein we have the fact clearly demonstrated that sodium carbonate will stimulate dilute pancreatic juice to increased proteolytic work.

I do not wish, however, to convey the idea that sodium carbonate has this power only in dilute solutions of pancreatic juice. I did not study the influence of sodium carbonate on the proteo-

TABLE F.—*Influence of Sodium Carbonate on the Proteolytic Action of Dilute Solutions of Pancreatic Juice.*

Number of experiment.	Duration in hours.	Fibrin in grams.	Sodium carbonate in grams.	Water in c.c.	Bile in c.c.	Puncreatic juice in c.c.	Loss of weight in grams.
I	4½	.501	0	20	0	.246	.054
	4½	.503	.050	20	0	.246	.065
	4½	.505	.100	20	0	.246	.068
	4½	.501	.050	20	.616	.246	.078
	4½	.501	.100	20	.616	.246	.062
II	4½	.502	0	20	0	.309	.076
	4½	.505	.050	20	0	.309	.127
	4½	.508	.100	20	0	.309	.133
	4½	.501	.050	20	.616	.309	.120
	4½	.504	.100	20	.616	.309	.125
III	4¾	.502	0	20	0	.492	.083
	4¾	.504	.025	20	.616	.492	.092
	4¾	.500	.050	20	.616	.492	.088
	4¾	.505	.025	20	0	.492	.096
	4¾	.505	.050	20	0	.492	.093
	4¾	.506	.100	20	0	.492	.097

lytic action of pancreatic juice under other conditions than those named in this table, and have, therefore, limited my conclusions to its influence on dilute solutions of the juice.

From all the evidence, presented in this paper, I am led to believe that the conditions which prevail throughout the entire small intestine in carnivorous animals are favorable to the proteolytic action of pancreatic juice. In these animals when the proteid food, partially saturated with hydrochloric acid, is discharged from the pylorus, it at once comes in contact with the mixture of bile and pancreatic juice, and immediately the trypsin finds itself under conditions most favorable for its proteolytic action. These conditions prevail for a short time only, when the pancreatic juice, more or less weakened by dilution, as it passes down the intestinal canal, is called upon to act in the presence of sodium carbonate in the lower and alkaline portion of the ileum; and here again we find these conditions very favorable for the proteolytic enzyme of the pancreas. If the above conditions, as assumed, be correct, then throughout the entire small intestine, trypsin finds itself under conditions more favorable to its proteolytic action than if it were acting, throughout the canal, upon neutral fibrin in neutral solution.

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V.—PANCREATIC DIGESTION FROM THE STANDPOINT OF THE COMPARATIVE ANATOMY OF THE BILE AND PANCREATIC DUCTS IN MAMMALS.

In 1891 I published a paper in the *Journal of Physiology* on "The Influence of Bile on the Fat-Splitting Properties of Pancreatic Juice." In this paper I demonstrated the important physiological fact that rabbits' bile expedites the fat-splitting properties of rabbits' pancreatic juice in the ratio of three to one. Reasoning by analogy from these experiments, I concluded that when the fresh bile of any animal is mixed with the fresh pancreatic juice of the same animal it will enable the pancreatic juice to split fats much more rapidly than if the juice were acting alone. If this inference be true it follows (since fat-splitting is a necessary preliminary step in fat-digestion) that all animals which have a common opening for their bile and pancreatic ducts are, by reason of this anatomical arrangement, much better endowed for the digestion of fats than those animals which do not have a common opening for these ducts. For these reasons one would expect to find, in all animals taking a quantity of fat in their food, such anatomical conditions as would provide for the preliminary mixing of bile and pancreatic juice before they commenced the work of fat-digestion.

In the same paper I further demonstrated that if fresh rabbits' bile be added to fresh rabbits' pancreatic juice, acting in the presence of a 0.25 per cent. solution of hydrochloric acid, the bile not only neutralizes the retarding influence of the hydrochloric acid on the fat-splitting properties of pancreatic juice, but it also acts more powerfully in hastening this action of pancreatic juice when it is combined with the hydrochloric acid than it does when acting alone. If this physiological fact may be applied to the study of fat-digestion in other animals, then one may infer

that the pancreatic digestion of fats in all animals is promoted by the presence of bile and a small percentage of hydrochloric acid. Following this argument one is led to conclude that, in animals taking a large quantity of fat in their food, the bile and pancreatic juice should not only be poured into the duodenum through a common opening, but this common opening should be near the pylorus so as to provide for the mixing of the bile and pancreatic juice with the acid contents from the stomach high up in the duodenum.

In this same paper, in which the importance of bile in fat-digestion was demonstrated, attention was called to the fact that the presence of a gall-bladder served an important purpose in fat-digestion, in that it supplied bile in large quantities and at the proper time to assist the pancreatic juice in splitting fats. Animals which take considerable fat in their food should therefore be provided with gall-bladders.

It was further demonstrated in this same paper that 0.25 per cent. of sodium carbonate (the amount which the succus entericus is supposed to contain) would greatly retard the fat-splitting properties of pancreatic juice. I therefore concluded that in animals taking considerable fat in their food the anatomical conditions should be such as to retard the rate of passage of food-stuffs through the duodenum, so that the fats may be exposed to the action of pancreatic juice in the duodenum long enough for the requisite amount of fat-splitting to be accomplished. Otherwise the food would be hastened along the intestines and into the presence of sodium carbonate, which would retard fat-splitting and consequently interfere with fat-digestion.

In 1899 I published a second paper in the *Journal of Physiology* on "The Influence of Bile, of Acids, and of Alkalies on the Proteolytic Action of Pancreatic Juice." In this paper I demonstrated that rabbits' bile, when added to rabbits' pancreatic juice, acting upon neutral fibrin, will markedly stimulate the proteolytic power of the juice; in most of the experiments the juice was able to do one-fourth more work in proteolysis by reason of the presence of the bile. From this physiological fact one may reason by analogy that the fresh bile of any animal will increase the proteolytic power of the fresh pancreatic juice of the same animal. If this reasoning be well founded, then one may conclude that the preliminary mixing of the bile and pancreatic juice

which occurs in some animals serves an important physiological purpose in proteid digestion, and that all animals taking a large quantity of proteids in their food should have such an anatomical arrangement of their bile and pancreatic ducts as will provide for the mixing of the bile and pancreatic juice either before or directly after they are brought into contact with the food. It follows also that these animals should be provided with gall-bladders, that the bile may be supplied in proper quantities and at the proper time to satisfy the demands of the pancreatic digestion of proteids.

In this same paper my experiments "led me to the conclusions that a small quantity of combined hydrochloric acid, not greater than one-half saturation, will but slightly diminish the proteolytic action of rabbits' pancreatic juice, and that when rabbits' bile is added to these mixtures the retarding influence of the hydrochloric acid is not only neutralized but an actual increase in proteolysis is attained." If this principle of proteid digestion holds good in all animals, then one would expect to find in proteid-eating animals that the bile and pancreatic juice was poured into the duodenum not only through a common opening, but that this common opening was near the pylorus.

In 1899 I published a paper in the *American Journal of Physiology* on "The Diastatic Action of Pancreatic Juice." From the experiments recorded in this paper the conclusion was drawn that bile plays a rather unimportant rôle in starch digestion. It is, in fact, not necessary to the diastatic action of pancreatic juice when the juice is acting in neutral or slightly alkaline solution, but the bile may be of great assistance to pancreatic juice—indeed, almost necessary to its full diastatic action—when the former is acting in the presence of free acid. It was also shown in this paper that both albumen and bile would, when combined with a small amount of hydrochloric acid, somewhat increase the diastatic action of pancreatic juice over the amount of work it could do in neutral solution. If these experiments may be taken as evidence of the unimportance of bile in the diastatic action of pancreatic juice in all animals, then one may conclude that no important physiological purpose would be served by the union of the bile and pancreatic ducts in animals purely herbivorous, and that there is no apparent physiological reason why these animals should be provided with gall-bladders.

In the above outline I have briefly reviewed a portion of the work which I have done during the past few years on the digestive action, on various food-stuffs, of rabbits' pancreatic juice acting under various conditions, and from these observations I have attempted to make out the anatomical conditions which would best serve the physiological purposes of pancreatic digestion in different animals. In following out this line of reasoning I have assumed that variations in the structure of the digestive organs of different animals had been determined by the physiological laws of food digestion. I have also assumed, and in this assumption there may be room for doubt, that the physiological laws of pancreatic digestion are the same in all animals.

The above observations, however, derive their importance not so much from the arguments which they contain as from the fact that they are to offer corroborative testimony to the physiological conclusions which will hereafter be drawn from anatomical data.

With this prelude I shall now attempt certain conclusions concerning the laws of food digestion, from a study of the comparative anatomy of the digestive organs of various animals. In this study I have classified all animals, according to the character of their food, as herbivorous, carnivorous, or omnivorous. It is assumed that the herbivorous animals partake chiefly of starch, the carnivorous and insectivorous chiefly of proteids and fats, and the omnivorous of a fair proportion of all these foods.

"In a comparative anatomy study of the kind here attempted, the difficulties are many and the liabilities to draw false conclusions from misinterpreted and incomplete anatomical data are very great. While one may assume without fear of contradiction that the various anatomical arrangements of the bile and pancreatic ducts in different species of mammals have a definite physiological significance, and that for the most part this anatomical arrangement has resulted, by natural selection, from the physiological laws controlling the digestion of food, yet even with this preface it is impossible for one to estimate in an accurate way the proportionate influence which the various physiological laws may have had in the development of the existing anatomical conditions. For example, one cannot say that such physiological conditions as the presence of a gall-bladder discharging its bile through a duct, common to it and the pancreas, into the duodenum in close proximity to the pylorus were developed in the car-

nivora exclusively as a result of the physiological laws of fat-digestion; nor can one say that the physiological laws of proteid digestion have exclusively determined these conditions. At best one can only approximately estimate, from existing physiological facts, the proportionate influence which either of these factors may have had in determining the various anatomical arrangements of the bile and pancreatic ducts in mammals.

"A source of error, in attempting to draw physiological conclusions from anatomical data, lies in the fact that there are always minor accessory anatomical conditions, the physiological importance of which it is difficult to estimate. For example, in the carnivora and many omnivora I have noted the following anatomical peculiarities of the duodenum: It has a horseshoe shape, its convexity looking downward; it gradually diminishes in caliber, being smallest at its junction with the jejunum; it is closely attached to the head of a fleshy pancreas and has a short mesenteric attachment, for which reasons it is much less movable than is this portion of the small intestine in the herbivora. These anatomical peculiarities of the duodenum in the carnivora and in some omnivora no doubt have a great influence in diminishing the rate of passage of food-stuffs through the duodenum, and in this way they increase the duration of the exposure of food to the combined action of the pancreatic juice and bile in the duodenum. But it is impossible to estimate what influence such accessory anatomical conditions as these may have (by modifying the influence of the physiological laws of food digestion) in determining the anatomical arrangements of the bile and pancreatic ducts in different species of mammals. Errors of inference from this cause must therefore remain in great part uncorrected.

"Another liability to error, in such a study as this, results from assuming that the same organs in different orders of mammals always do the same kind of work. For example, the stomachs of the carnivora and of the herbivora are very different organs, and are capable of very different kinds of physiological work. And here again it is not possible to estimate the influence which differing anatomical conditions, in other portions of the digestive tract, may have had in modifying the influence which the duodenal digestion of food-stuffs has had in determining the various arrangements of the bile and pancreatic ducts in different mammals.

"Another and most important cause of error, in making physiological deductions from comparative anatomy data, is that the primitive type from which the animal is descended is an influential factor in determining structure in all subsequent generations. For example, the seal and the porpoise are descended from very differently constructed ancestors, and this fact no doubt accounts for the presence of a gall-bladder in the seal and its absence in the porpoise, notwithstanding the fact that for numberless generations both have been purely fish-eaters."

Notwithstanding these difficulties, I shall attempt to make physiological deductions from such comparative anatomy data as I have been able to collect. The following tables I have taken from a former article of mine published in this journal in December, 1895, and due acknowledgement is hereby made not only for these tables, but for the above quotation.

QUADRUMANA.

Species and Name of Animal.	Common opening of bile and pancreatic ducts is from pylorus:	Presence of gall-bladder.	Gall-duct opening is distant from pylorus:	Food of animal.
Man	8 cm.	Yes.	8 cm.	Omnivorous.
Troglodyte Gorilla	25 cm.	Yes.	25 cm.	Herbivorous.
Chimpanzee (*Troglodytes niger*)	15 to 20 cm.	Yes.	15 to 20 cm.	Herbivorous.
Common Macaque Monkey (*Macacus cynomolgus*)	3 cm.	Yes.	3 cm.	Omnivorous.
Capuchin Monkey (*Cebus capucinus*)	2 cm.	Yes.	2 cm.	Insectivorous.
Lemur, nocturnal (*Indris brevicaudatus*)	3 cm.	Yes.	3 cm.	Insect. chiefly.
Lemur, diurnal (*Lemur mongoz*)	$7\frac{1}{2}$ cm.	Yes.	$7\frac{1}{2}$ cm.	Omnivorous.
Lemur, ruffed (*Lemur varius*)	$1\frac{1}{4}$ cm.	Yes.	$1\frac{1}{4}$ cm.	Insect. chiefly.

This table contains three insectivorous animals, namely, the Capuchin monkey, the nocturnal lemur, and the ruffed lemur. It will be observed that in these animals the common openings of the bile and pancreatic ducts are placed very near the pylorus. If this location of the common duct be compared with its location in the herbivorous gorilla and chimpanzee, which belong to this same species, the contrast is very striking. In the gorilla the common duct is located twenty-five centimetres from the pylorus, and in the ruffed lemur it approaches as near as one and a fourth centimetres. If one assumes that the anatomical conditions as they occur in these animals serve the best purposes of food digestion, then one must infer that this anatomical arrangement which gives to insectivorous animals a common opening for their bile

and pancreatic ducts and places this common opening near the pylorus serves an important physiological purpose in the digestion of fats and proteids—the chief food-stuffs of these animals. From these facts one may infer that the preliminary mixing of bile and pancreatic juice before they enter the duodenum is of physiological importance in the digestion of fats and proteids, and this inference will be strengthened into a conclusion by a study of subsequent tables. From this table one may also infer that this preliminary mixing of bile and pancreatic juice is of importance in the digestion of starchy foods, since both the herbivorous chimpanzee and gorilla have a common opening for the bile and pancreatic ducts; but a study of subsequent tables will show that this inference is fallacious. An important physiological inference, however, which may be made from the above table is that the pancreatic digestion of fats and proteids as it occurs in insectivora is facilitated by bringing the bile and pancreatic juice in contact with the food very soon after it leaves the stomach, and that the pancreatic digestion of starches as it occurs in herbivora is not facilitated by bringing these juices in contact with the food high up in the duodenum. If one remembers that the duodenal contents, in the carnivora, are acid, and that the food as it leaves the stomach contains more or less combined hydrochloric acid, one is led to the inference that the pancreatic digestion of fats and proteids is facilitated in the insectivora not only by the bile, but also by such acids as may be combined with the food-stuffs as they enter the duodenum. If these inferences be not true, then no apparent physiological purpose is served by placing the common bile and pancreatic ducts near the pylorus in the Capuchin monkey, nocturnal lemur, and ruffed lemur, the three insectivora in the above table.

These inferences concerning the laws which govern the digestion of fats and proteids in the insectivora are of more value as they are compared with the inferences made from the same table concerning the pancreatic digestion of starches in the herbivorous chimpanzee and gorilla. If one may assume that starch digestion in these animals is facilitated by the anatomical arrangement which pours the bile and pancreatic juice into the duodenum twenty to twenty-five centimetres below the pylorus (since the lower portion of the duodenum is alkaline in the herbivora), then will follow the inference that pancreatic juice assisted by the bile

can do its best diastatic work in a feebly alkaline medium. If, on the other hand, an acid medium is much more favorable to the diastatic action of pancreatic juice than an alkaline, then would the bile and pancreatic ducts be placed in the herbivora, as they are in the carnivora, high up in the duodenum near the pylorus. The inference therefore seems plain that an acid medium has no advantages over an alkaline medium in the pancreatic digestion of starches. That the pancreatic juice prefers an alkaline medium for the digestion of starches is also indicated by the fact, above referred to, that in the herbivora the intestinal contents lose their acidity sooner and become more alkaline than in the carnivora, and also by the fact that the bile of herbivorous animals has a greater degree of alkalinity than the bile of the carnivora.

If one again refers to the table it will be seen that the omnivorous animals of this species, viz., man and the diurnal lemur, have the common opening of their bile and pancreatic ducts not so near the pylorus as the carnivora nor so far away as the herbivora, which would indicate that nature had compromised on this mid-distance as between the extremes demanded by the laws of food digestion in the purely carnivorous and herbivorous animals.

CHEIROPTERA.

Species and Name of Animal.	Common opening of bile and pancreatic ducts is from pylorus:	Presence of gall-bladder.	Gall-duct opening is distant from pylorus:	Food of animal.
Fruit Bat (*Pteropus Edwardsii*)....	12½ cm.	Yes.	12½ cm.	Herbivorous.
Insect Bat (*Vespertilio noctula*)....	½ cm.	Yes.	½ cm.	Insectivorous.

This table is here inserted in support of the above inferences drawn from the Quadrumana. The contrast is very striking between the location of the openings of the common bile and pancreatic ducts in the fruit-eating and insect-eating bat. The insectivorous bat, which takes a large quantity of fats and proteids in its food, has the common opening of bile and pancreatic ducts immediately below the pylorus, where these juices may come in contact with the acid food-stuffs as they leave the stomach; while in the fruit bat this opening is twelve and one-half centimetres from the pylorus. The marked difference that here exists in the location of these ducts emphasizes the great advantage in food digestion which must accrue to the carnivorous

animals in having the common opening of the bile and pancreatic ducts in close proximity to the pylorus, and it also emphasizes the fact, above noted, that this anatomical arrangement is of no physiological advantage to herbivorous animals, otherwise the bile and pancreatic juice would enter the duodenum of the fruit bat, as it does in the insect bat, near the pylorus, instead of twelve and a half centimetres below.

CARNIVORA.

Species and Name of Animal.	Common opening of bile and pancreatic ducts is from pylorus:	Distance between bile and pancreatic ducts.	Presence of gall-bladder.	Gall-duct opening is distant from pylorus:	Food of animal.
Lion (*Felis leo*)	6 cm.	.....	Yes.	6 cm.	Carnivorous.
Tiger (*Felis tigris*)	5 cm.	.....	Yes.	5 cm.	Carnivorous.
Leopard (*Felis Leopardus*)	4 cm.	.....	Yes.	4 cm.	Carnivorous.
Domestic Cat (*Felis domestica*)	2½ cm.	.....	Yes.	2½ cm.	Carnivorous.
Wildcat (*Felis Lynx rufus*)	3 cm.	.....	Yes.	3 cm.	Carnivorous.
Lynx (*Felis Lynx canadensis*)	Near.	.....	Yes.	Near.	Carnivorous.
Panther (*Felis concolor*)	Near.	.....	Yes.	Near.	Carnivorous.
Coyote, American (*Canis latrans*)	.....	3.8 cm.	Yes.	2.6 cm.	Carnivorous.
Wolf (*Canis lupus*)	Near.	On same level.	Yes.	Near.	Carnivorous.
Fox, African (*Canis Vulpes niloticus*)	.....	5½ cm.	Yes.	½ cm.	Omnivorous.
Fox, European (*Canis Vulpes vulgaris*)	.....	5½ cm.	Yes.	Near.	Omnivorous.
Dog, Japanese (*Canis Nyctereutes procyonides*)	.....	5½ cm.	Yes.	3 cm.	Omnivorous.
Dog, domestic (*Canis familiaris*)	5 cm.	Acces. pan. duct, from pyl. 7.5 cm.	Yes.	5 cm.	Omnivorous.
Skunk (*Mephitis mephitica*)	2½ cm.	.....	Yes.	2½ cm.	Carn. chiefly.
Badger (*Meles vulgaris*)	.....	2 cm.	Yes.	.....	Omnivorous.
Brown Bear (*Ursus arctus*)	Acces. pan. duct.	Above bile duct.	Yes.	.....	Omnivorous.
Raccoon, Albino (*Ursus Procyon lotor*)	4 cm.	.....	Yes.	4 cm.	Omnivorous.
Coati, Mexican (*Ursus Nasua narica*)	3 cm.	.....	Yes.	3 cm.	Insectiv. etc.
Hedgehog (*Insectivora Erinaceus europæus*)	2 cm.	.....	Yes.	2 cm.	Insectivorous.

It will be noted that twelve of the nineteen animals in the above table are carnivorous, and that in all of these carnivorous animals, excepting the American coyote, the bile and pancreatic juice are poured into the duodenum in close proximity to the pylorus either through a common opening or through separate openings entering the duodenum on the same level, thus providing for the mixing of the bile and pancreatic juice immediately they are poured into the duodenum. Even in the American coyote the distance—three and one-half centimetres—between the openings of the bile and pancreatic ducts is so short that it

may hardly be considered as an exception to the rule that carnivorous animals have an anatomical arrangement which provides for the mixing of bile and pancreatic juice before, or directly after, their entrance into the intestine. It is interesting to note in this table that in the large animals like the lion, the tiger, and the leopard, which are exclusively carnivorous, the common bile and pancreatic duct approaches so closely to the pylorus (from four to six centimetres) that one must believe there is decided physiological advantage in this arrangement which provides that the bile and pancreatic juice shall come in contact with acid food-stuffs immediately after they are discharged from the stomach. It appears, in fact, from a study of the comparative anatomy of the bile and pancreatic ducts in all the herbivorous, omnivorous, and carnivorous animals in the tables here presented that one may formulate the rule that *the more strictly carnivorous the animal, the more closely will the opening of the common bile and pancreatic duct approach the pylorus.* These facts surely justify us in concluding that the pancreatic digestion of fats and proteids is facilitated by the presence of bile and combined acids to the extent that they exist in food-stuffs high up in the duodenum, and warrant us in concluding that the fundamental principles which govern fat and proteid digestion in the carnivora are the same as in the rabbit, since, as noted in the preface, laboratory experiments with the bile and pancreatic juice of this latter animal lead to the same physiological conclusions.

In the above table, however, special interest attaches to a study of the arrangement of the bile and pancreatic ducts in the seven omnivorous animals. Here one finds that the African fox, the European fox, the Japanese dog, and the badger have separate openings for their bile and pancreatic ducts. In the first three the pancreatic juice is poured into the duodenum five and one-half centimetres below the opening of the bile duct. In the other three omnivorous animals in this table, namely, the domestic dog, the brown bear and the raccoon, one finds in two of these animals an accessory pancreatic duct opening below the common duct; and in only one, the raccoon, do we find all of the bile and pancreatic juice poured through a common opening into the duodenum in close proximity to the pylorus. It is quite evident, therefore, from a study of this table that in omnivorous animals there is less physiological necessity than in the carnivora

for the preliminary mixing of the bile and pancreatic juice before they come in contact with food-stuffs. The physiological importance of this variation will appear in the following table:

PACHYDERMATA.

Species and Name of Animal.	Common opening of bile and pancreatic ducts is from pylorus:	Distance between bile and pancreatic ducts.	Presence of gall-bladder.	Gall-duct opening is distant from pylorus.	Food of animal.
Horse (*Equus caballus*)	15 cm.	.....	No.	15 cm.	Herbivorous.
Ass (*Equus asinus*)	15 cm.	.....	No.	15 cm.	Herbivorous.
Elephant (*Elephas indicus*)	10 cm.	Accessory duct 5 cm. lower.	No.	10 cm.	Herbivorous.
Tapir (*Tapirus americanus*)	.....	Same level, common opening.	No.	.....	Herbivorous.
Pig (*Sus familiaris*)	.....	10 to 20 cm.	Yes.	2 to 5 cm.	Omnivorous.
Rhinoceros (*Rhinoceros unicornis*)	15 cm.	Same level.	No.	15 cm.	Herbivorous.
Hyrax (*Hyrax capensis*)	2.5 cm.	One 2 cm. lower.	No.	2.5 cm.	Herbivorous.

In this table we have emphasized the unimportance of the gall-bladder in herbivorous animals. The omnivorous pig is the only animal in the table provided with a gall-bladder. In all of the six herbivorous animals it is absent. The absence of the gall-bladder in these herbivorous animals clearly indicates the unimportance of this organ in starch digestion. The presence of a gall-bladder in all carnivorous animals indicates that there is a physiological necessity for a reservoir of this kind for holding the bile so that it can be discharged in quantity into the duodenum during the pancreatic digestion of fats and proteids. That this reservoir for bile is unnecessary in herbivorous or starch-eating animals is clearly proven by the fact that it gradually diminishes in size as the animal takes less fat and proteid in its food, till in some of the exclusively herbivorous animals, as noted in the above table, it is absent. Clearly, then, the character of the food in different species of animals determines the presence and size of the gall-bladder. In the carnivora the gall-bladder is large, and in the herbivora it is small or altogether absent, while in the omnivora it is intermediate in size. The physiological inference from these facts is that bile does not serve any very important purpose in the pancreatic digestion of starches, for certainly nothing short of the unimportance of bile in the pan-

creatic digestion of starches would account for the gradual diminution in size and final loss of the gall-bladder and a consequent inability to regulate the flow of bile according to the needs of pancreatic digestion in animals as they become more and more exclusively herbivorous. This physiological inference corrects the physiological inference made from the Quadrumana, that "bile is of importance in the pancreatic digestion of starches." Evidently this inference was made from incomplete anatomical data. The inference, however, just drawn from the above table, that bile is of little value to pancreatic juice in the digestion of starches, is made stronger by a study of subsequent tables, and is, moreover, in accord with the physiological conclusion, drawn from laboratory experimentation, and outlined in the preface to this paper, that "bile has little if any favorable action on the diastatic action of pancreatic juice."

In the study of the above table it will be noticed that there is a tendency to the separation of the bile and pancreatic ducts in herbivorous animals. In many of these animals the opening of the pancreatic duct is some distance below the bile duct. This is a fact in comparative anatomy of no little importance. This separation of bile and pancreatic ducts as noted in the last table occasionally occurs in the omnivora, but it never occurs in the carnivora. In these last named animals the bile and pancreatic ducts enter the duodenum by a common opening, except in a few instances where they enter on a common level, thus providing for the mixing of bile and pancreatic juice before or directly after they enter the duodenum. These facts clearly indicate that the preliminary mixing of bile and pancreatic juice is unimportant in the herbivora. It is also clear from a study of all the tables here presented that the union or separation of the bile and pancreatic ducts, as well as the location of the openings of these ducts, with reference to the pylorus, have been determined in different animals by the character of their food-stuffs. The carnivora have a common opening near the pylorus. The herbivora either have a common opening far from the pylorus, or the ducts are separated and the pancreatic opening is far from the pylorus, while the gall duct may remain near the pylorus. The omnivora present an anatomical arrangement of these ducts which is a compromise between the extremes as found in the carnivora and herbivora. From these facts the physiological inference may be

drawn that bile is not necessary to the pancreatic digestion of starches. This inference accords with that above drawn from the comparative anatomy of the gall-bladder in these animals, as well as that drawn from chemical experiments made with rabbits' bile and pancreatic juice as outlined in the preface.

MARSUPIALIA.

Species and Name of Animal.	Common opening of bile and pancreatic ducts is from pylorus:	Presence of gall-bladder.	Gall-duct opening is distant from pylorus:	Food of animal.
Dasyure (*Dasyurus viverrinus*)	2.5 cm.	Yes.	2.5 cm.	Carn.& insect.
Opossum (*Didelphys virginiana*)	2.5 cm.	Yes.	2.5 cm.	Carn.& insect.
Bandicoot (*Perameles nasula*)	2.5 cm.	Yes.	2.5 cm.	Insect. & herb.
Kangaroo (*Macropus giganteus*)	15 to 25 cm.	Yes.	15 to 25 cm.	Herbivorous.

In this table we have three insectivorous animals and one herbivorous, and the contrast in the arrangement of the bile and pancreatic ducts in these animals is even more striking than in the previous tables. The insectivorous dasyure, opossum and bandicoot have the common opening of their bile and pancreatic ducts within two and one-half centimetres of the pylorus, while the herbivorous kangaroo has the common opening of these ducts from fifteen to twenty-five centimetres below the pylorus. Here again we have emphasized the physiological necessity in the carnivora of mixing the bile and pancreatic juice high up in the duodenum, and the physiological importance in the herbivora of removing the pancreatic duct some distance from the pylorus.

PINNIPEDIA.

Species and Name of Animal.	Common opening of bile and pancreatic ducts is from pylorus:	Presence of gall-bladder.	Gall-duct opening is distant from pylorus:	Food of animal.
Seal (*Phoca vitulina*)	6.5 cm.	Yes.	6.5 cm.	Carnivorous.
Sea Bear (*Otaria ursina*)	5 cm.	Yes.	5 cm.	Carnivorous.

In the two carnivorous animals of this species we have the same arrangement of the bile and pancreatic ducts which we have noted in the carnivorous animals in the previous tables, and the same inferences may be drawn.

In the two herbivorous animals next presented one notes, in the small gall-bladders, an effort to get rid of this organ, which is of little use in herbivorous animals, and in the location of the

SIRENIA.

Species and Name of Animal.	Distance between bile and pancreatic ducts.	Presence of gall-bladder.	Gall-duct opening is distant from pylorus:	Food of animal.
Manatee (*Manatus latirostris*).....	Same level.	Yes.	13 cm.	Herbivorous.
Dugong (*Halicore Dugong*).....	Same level.	Yes.	12 cm.	Herbivorous.

pancreatic duct some distance from the pylorus one notes the arrangement commonly found in herbivora.

RODENTIA.

Species and Name of Animal.	Common opening of bile and pancreatic ducts is from pylorus:	Distance between bile and pancreatic ducts.	Presence of gall-bladder.	Gall-duct opening is distant from pylorus:	Food of animal.
Rabbit (*Lapus cuniculus*).....	.....	30 to 40 cm.	Yes (small).	1¼ cm.	Herbivorous.
Rat (*Mus decumanus*).....	2.5 cm.	.....	No.	2.5 cm.	Omnivorous.
Beaver (*Castor fiber*).....	.....	45.5 cm.	Yes.	7.5 cm.	Herbivorous.
Porcupine (*Spingurus prehensilis*)	.....	5⅝ cm.	Yes.	1 cm.	Herbivorous.

The omnivorous rat has no gall-bladder. This, from the point of view presented in this paper, is a decided disadvantage in the pancreatic digestion of fats and proteids. This disadvantage, however, is offset in the rat by an anatomical arrangement which pours the bile and pancreatic juice through a common opening in very close proximity to the pylorus. The rabbit, the beaver and the porcupine, on the other hand, all have gall-bladders, which, from the argument presented in this paper, they have little use for. The gall-bladders of these animals, however, are small, and will perhaps in time be altogether lost. The beaver and the rabbit, however, emphasize the apparent physiological importance to the herbivora of removing the opening of the pancreatic duct far from the pylorus. The beaver has the distinction of having the distance between the openings of its bile and pancreatic ducts greater than that of any other animal noted in these tables; and the rabbit, with the opening of its pancreatic duct from thirty to forty centimetres below the opening of the bile duct, enjoys the distinction of being in this regard second only to the beaver. From these facts it appears that while the physiological laws of food digestion in herbivorous animals may demand that the pan-

creatic juice be poured into the duodenum some distance below the pylorus, this demand does not carry with it the necessity that the bile duct should enter at this same low level. The fact, however, that in many herbivora the bile and pancreatic juice are poured through a common opening into the duodenum proves that bile, while it may not increase, certainly does not retard the diastatic action of pancreatic juice; this physiological inference is in accord with that drawn from laboratory study of the diastatic action of rabbits' pancreatic juice as outlined in the preface to this paper.

RUMINANTIA.

Species and Name of Animal.	Common opening of bile and pancreatic ducts is from pylorus:	Distance between bile and pancreatic ducts.	Presence of gall-bladder.	Gall-duct opening is distant from pylorus:	Food of animal.
Ox (*Bos taurus*)	.....	35 to 50 cm.	Yes.	25 to 30 cm.	Herbivorous.
Goat (*Capra hircus*)	30 to 40 cm.	.....	Yes.	30 to 40 cm.	Herbivorous.
Pygmy Deer, Malay (*Tragulus Stanleganus*)	12.2 cm.	.....	Yes.	12.2 cm.	Herbivorous.
Sheep (*Ovis aries*)	30 to 40 cm.	.....	Yes.	30 to 40 cm.	Herbivorous.
Giraffe (*Camelopardalis Giraffa*)	25 cm.	.....	No (2 to 1).	25 cm.	Herbivorous.
Camel (*Camelus bactrianus*)	58 cm.	.....	No.	58 cm.	Herbivorous.
Dromedary (*Camelus dromedarius*)	28 cm.	.....	No.	28 cm.	Herbivorous.

In this table we have seven herbivorous animals, all of whom have the opening of their pancreatic ducts far below their pylorus. In all of these animals the primitive type has maintained the union between the bile and pancreatic ducts, and placed them together many centimetres below the pylorus. In the ox, however, this union of the bile and pancreatic ducts is lost, and the opening of the pancreatic duct is carried far below that of the bile duct and the pylorus. These facts offer corroborative testimony to the physiological conclusions drawn from the study of the herbivorous animals in the preceding tables. The uselessness of the gall-bladder to herbivora is here again emphasized by its absence in the giraffe, camel and dromedary, for surely the disappearance of an organ is proof sufficient that it served no important physiological purpose. In the camel the gall-bladder is still occasionally found. In three dissections made by Dr. J. B. S. Jackson it was absent in two and present in one.

EDENTATA.

Species and Name of Animal.	Common opening of bile and pancreatic ducts is from pylorus:	Presence of gall-bladder.	Gall-duct opening is distant from pylorus:	Food of animal.
Armadillo (*Dasypus gigas*)	5 cm.	Yes.	5 cm.	Omnivorous.
Small Ant-eater (*Myrmecophaga didactylus*)	1¾ cm.	Yes.	1¾ cm.	Insectivorous.
Tamandura Ant-eater (*Myrmecophaga tamanduris*)	5 cm.	Yes.	5 cm.	Insectivorous.
Cape Ant-eater (*Orycteropus capensis*)	2 cm.	Yes.	2 cm.	Insectivorous.
Two-toed Sloth (*Cholæpus didactylus*)	.....	No.	10 cm.	Herbivorous.
Three-toed Sloth (*Bradypus tridactylus*)	.....	Small.	2.5 cm.	Herbivorous.

In this table we have two herbivorous animals—one, the two-toed sloth, has no gall-bladder, and the other, the three-toed sloth, has a small gall-bladder. This is in contrast with the insectivorous and omnivorous animals of this species, all of which have gall-bladders. It appears, therefore, not only from this table, but from all previous tables, that the gall-bladder is disappearing in herbivorous animals, and that the influence of the primitive type is in great part responsible for the presence of the gall-bladder in these animals.

MONOTREMATA.

Species and Name of Animal.	Common opening of bile and pancreatic ducts is from pylorus:	Presence of gall-bladder.	Gall-duct opening is distant from pylorus:	Food of animal.
Duckbill (*Ornithorhynchus paradoxus*)	2 cm.	Large.	2 cm.	Insectivorous.
Australian Hedgehog (*Echidna hystrix*)	3 cm. or same level.	Large.	2 cm.	Insectivorous.

The large gall-bladders and common openings of the bile and pancreatic ducts near the pylorus in these animals support the conclusions previously drawn from the study of the carnivora in the preceding tables.

In the following table we have presented three carnivorous animals, in all of which the gall-bladder is absent. This is a most important exception, since the animals of this species are the only carnivorous animals (presented in these tables) not having gall-bladders. The absence of the gall-bladder in these animals is no doubt a disadvantage to them in food digestion; but nature apparently attempts to right this defect by giving to

CETACEA.

Species and Name of Animal.	Common opening of bile and pancreatic ducts is from pylorus:	Presence of gall-bladder.	Gall-duct opening is distant from pylorus:	Food of animal.
Whale (*Physeter macrocephalus*)....	Immediately below.	No.	Immediately below.	Carnivorous.
Dolphin (*Delphinus delphis*).....	Immediately below.	No.	Immediately below.	Carnivorous.
Porpoise (*Phocæna communis*)..... ..	Immediately below.	No.	Immediately below.	Carnivorous.

these animals a common bile and pancreatic duct opening immediately below the pylorus. The absence of gall-bladders in whales is therefore an exception which proves the rules that the physiological laws of food digestion in the carnivora demand, first, that they shall have a gall-bladder; secondly, that they shall have a common opening for the bile and pancreatic ducts; thirdly, that this common opening of the bile and pancreatic ducts shall be placed high up in the duodenum close to the pylorus, to provide for the mixing of these juices with the food-stuffs soon after they are discharged from the stomach.

In the whales, however, the physiological laws of food digestion have failed to overcome the influence of the primitive type in determining structure, and have not for this reason been able to develop gall-bladders. These laws, however, failing in this particular have placed the common opening of the bile and pancreatic ducts in these animals almost immediately below the pylorus; in this arrangement apparently making an effort to overcome the disadvantages from which these animals suffer by reason of the absence of gall-bladders.

In conclusion I desire to call attention to certain important facts in the comparative anatomy of the small intestine which do not appear in the above tables, and yet are of considerable physiological importance to the discussion of the questions considered in this paper. These facts relate to the influence which the shape and mobility of the duodenum may have on the rate of passage of food-stuffs along this portion of the alimentary canal. They are as follows:

The duodenum in the carnivora is more closely attached to the head of a fleshy pancreas and has a shorter mesenteric attachment than it has in the herbivora. These facts make peristaltic and other movements less active in the duodenum of the carniv-

ora than in the herbivora, and therefore cause the food to remain longer in the duodenum of the carnivora than in the herbivora. The horseshoe shape of the duodenum in the carnivora may also contribute to this same end. It appears, therefore, that certain anatomical conditions have been developed in the carnivora which have the effect of so slowing the rate of passage of food through the duodenum that the time of exposure of food-stuffs in the duodenum of these animals to the action of bile and pancreatic juice is greater than in the herbivora.

From these facts one would infer that it is of physiological importance that the fats and proteids should be retarded in their passage through the duodenum in order that they may be longer acted upon by the bile and pancreatic juice while the food yet contains combined acid, and before it reaches the alkaline succus entericus of the jejunum and ileum. This physiological inference finds support in laboratory experiments which I have previously published, and have outlined in the preface to this paper.

It is a well-known fact that climate may have considerable influence in determining the amount of fat and proteid taken. Arctic animals take much more fat than animals of the same family living in the tropics. In view of this fact, it would be interesting to inquire whether a long residence of many generations in a hot or a cold climate does, by reason of the difference in the amount of fat and proteid taken, produce a change in the arrangement of the bile and pancreatic ducts in animals of *the same family*, despite the potent influence which the primitive type would have in maintaining the same structure under all conditions. For example, it would be interesting to know whether there is any difference in the arrangement of the bile and pancreatic ducts in the arctic and the tropic representatives of the dog and bear families, and it would also be worth while to compare the anatomy of the bile and pancreatic ducts of the Esquimaux with that of human natives of the tropics. But for want of reliable data this interesting question, of the influence of climate on the anatomy of these ducts, could not be considered in this study.

With the assistance of Dr. Frank Southgate, I made the measurements in the following named animals: Chimpanzee, leopard, raccoon, lemur, Capuchin monkey, seal, wildcat, domes-

tic cat, Macaque monkey, opossum, dog, rat, ox, sheep, pig, rabbit and skunk.

I am also indebted for assistance in the preparation of the tables to the following named gentlemen: Dr. Leitz, of Frankfort-on-the-Main; Prof. A. Tarenetsky, of St. Petersburg; Prof. T. Munk, of Berlin; Prof. Pietro Albertoni, of Bologna.

I also obtained much valuable information from the lectures of Wm. Henry Flower, published in the *Medical Times and Gazette*, London, 1872, as well as from the works of Owen, Cuvier and M. Edwards.

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VI.—PANCREATIC DIGESTION OF CASEIN.[1]

In the following experiments, which were devised for the purpose of studying certain phases of the pancreatic digestion of casein, I used rabbits' pancreatic juice obtained by the method I have elsewhere described.[2] Pancreatic juice, thus obtained, was collected in a common receptacle and afterward equally divided between the digestion tubes of an experiment, so that each tube might contain an equal quantity of pancreatic juice of like digestive capacity.

The bile was also obtained from the rabbit and filtered before using. The milk employed was ordinary dairy milk, boiled and neutralized.

Each digestion tube of an experiment contained the same quantity of this milk, diluted either with an equal quantity of water or some other diluent, as detailed in the various experiments.

The digestion tubes were kept in a water bath, at a temperature of 38° C., for five or six hours, and their contents were stirred from time to time with glass rods especially prepared for the purpose. At the close of each experiment the undigested casein in each tube was coagulated by the addition of lactic acid and a saturated solution of ammonium sulphate. By filtration, in a warm chamber, this undigested casein was received on weighed and marked filter papers, which, after being thoroughly washed, was slowly dried and weighed at a temperature of 100° C. The amount of undigested casein in each tube was obtained by subtracting from the gross weight thus obtained the weight of the corresponding filter paper. Tube No. 1 of each experiment contained the same quantity of milk as the other tubes, but did

1 Read before the American Pediatric Society, Washington, D. C., May 1-3, 1900.

2 *American Journal of Physiology*, Vol. ii, No. 5.

not contain pancreatic juice or other ingredients which might change the casein. At the close of an experiment, therefore, tube 1 contained unchanged casein which, when coagulated, was used to determine the amount of casein each tube contained at the beginning of the experiment. The amount of casein which had been converted into peptones in each tube was obtained by subtracting from the amount of casein in tube 1 the amount of undigested casein in each of the subsequent tubes.

It will be noted that 15 cubic centimetres of the different specimens of milk used in the various experiments did not always contain the same amount of casein, and it is for this reason that the corresponding tubes of different experiments cannot be compared with one another. The comparative accuracy, however, of the deductions drawn from a comparison of the various tubes

EXPERIMENT I.—TIME, 6 HOURS.

Contents of Tubes.	Pancreatic Juice. Minims.	Bile. Minims.	Undigested Casein. Grams.	Digested Casein. Grams.	Tube Numbers.
Milk, 15 c.c..... Water, 15 c.c.....	0	0	1.174	0	1
Milk, 15 c.c..... Water, 15 c.c.....	6	0	.620	.554	2
Milk, 15 c.c..... Water, 15 c.c.....	6	0	.640	.534	3
Milk, 15 c.c..... Water, 15 c.c.....	6	12	.593	.581	4
Milk, 15 c.c..... Water, 15 c.c.....	6	12	.590	.584	5
Milk, 15 c.c..... Water. 15 c.c ..... HCl Dilute, m. ½.....	6	12	.580	.594	6
Milk, 15 c.c..... Water, 15 c.c..... HCl Dilute, m. ½.....	6	12	.584	.590	7
Milk, 15 c.c..... Water, 15 c.c..... HCl Dilute, m. 1.....	6	12	.607	.567	8
Milk, 15 c.c..... .4 per cent. Sol. Sodium Carbonate, 15 c.c.....	6	0	.532	.642	9
Milk, 15 c.c..... .8 per cent. Sol. Sodium Carbonate, 15 c.c.....	6	0	.521	.653	10
Milk, 15 c.c..... Lime Water, 15 c.c.....	6	0	.522	.652	11

of an experiment is assured by the fact that the same quantity of the same milk was used in each tube of an experiment.

The maltose solution used in these experiments was prepared by subjecting a mixture of water and one of the Liebig foods to the action of a diastase for one hour. At the end of this time, the diastatic ferment was destroyed by boiling and the maltose solution filtered through ordinary filter paper.

By the above method the preceding and following experiments were made.

EXPERIMENT II.—TIME, 6 HOURS.

Contents of Tubes.	Pancreatic Juice. Minims.	Bile. Minims.	Undigested Casein. Grams.	Digested Casein. Grams.	Tube Numbers.
Milk, 15 c.c. Water, 15 c.c.	0	0	1.125	0	1
Milk, 15 c.c. Water, 15 c.c.	5	0	.697	.428	2
Milk, 15 c.c. Lime Water, 15 c.c.	5	0	.640	.485	3
Milk, 15 c.c. Lime Water, 15 c.c.	5	10	.583	.542	4
Milk, 15 c.c. .4 per cent. Sol. Sodium Carbonate, 15 c.c.	5	0	.584	.541	5
Milk, 15 c.c. .4 per cent. Sol. Sodium Carbonate, 15 c.c.	5	10	.540	.585	6
Milk, 15 c.c. Water, 15 c.c. HCl Dilute, m. ½	5	10	.598	.527	7
Milk, 15 c.c. Water, 15 c.c. HCl Dilute, m. ½	5	10	.606	.519	8
Milk, 15 c.c. Water, 15 c.c. HCl Dilute, m. 1	5	10	.660	.465	9
Milk, 15 c.c. 2 per cent. Sol. of Milk Sugar, 15 c.c.	5	10	.651	.474	10
Milk, 15 c.c. Maltose Solution, 15 c.c.	5	10	.690	.435	11
Milk, 15 c.c. Maltose Solution, 15 c.c.	5	10	.639	.486	12

EXPERIMENT III.—TIME, 5 HOURS.

Contents of Tubes.	Pancreatic Juice. Minims.	Bile. Minims.	Undigested Casein. Grams.	Digested Casein. Grams.	Tube Numbers.
Milk, 15 c.c Water, 15 c.c	0	0	1.039	0	1
Milk, 15 c.c Water, 15 c.c	6	8	.581	.458	2
Milk, 15 c.c Water, 15 c.c HCl Dilute, m. ½	6	8	.531	.508	3
Milk, 15 c.c Water, 15 c.c HCl Dilute, m. 1	6	8	.579	.460	4
Milk, 15 c.c Water, 15 c.c HCl Dilute, m. ½	6	8	.495	.544	5
Milk, 15 c.c Water, 15 c.c HCl Dilute, m. 1	6	8	.629	.410	6

EXPERIMENT IV.—TIME, 5 HOURS.

Contents of Tubes.	Pancreatic Juice. Minims.	Bile. Minims.	Undigested Casein. Grams.	Digested Casein. Grams.	Tube Numbers.
Milk, 15 c.c Water, 15 c.c	0	0	1.115	0	1
Milk, 15 c.c Water, 15 c.c	10	0	.425	.690	2
Milk, 15 c.c Water, 15 c.c HCl Dilute, m. ½	10	0	.435	.680	3
Milk, 15 c.c Water, 15 c.c HCl Dilute, m. 1	10	0	.588	.527	4
Milk, 15 c.c Water, 15 c.c HCl Dilute, m. ½	10	0	.518	.597	5
Milk, 15 c.c Water, 15 c.c HCl Dilute, m. 1	10	0	.596	.519	6
Milk, 15 c.c .4 per cent. Sol. Sodium Carbonate, 15 c.c	10	0	.418	.697	7

EXPERIMENT V.—TIME, 5 HOURS.

Contents of Tubes.	Pancreatic Juice. Minims.	Bile. Minims.	Undigested Casein. Grams.	Digested Casein. Grams.	Tube Numbers.
Milk, 15 c.c. Water, 15 c.c.	0	0	1.080	0	1
Milk, 15 c.c. Water, 15 c.c.	8	10	.570	.510	2
Milk, 15 c.c. Water, 15 c.c. HCl Dilute, m. ½	8	10	.510	.570	3
Milk, 15 c.c. Water, 15 c.c. HCl Dilute, m. ½	8	10	.482	.598	4
Milk, 15 c.c. Lime Water, 15 c.c.	8	10	.505	.575	5
Milk, 15 c.c. 2 per cent. Sol. of Milk Sugar, 15 c.c.	8	10	.551	.529	6

EXPERIMENT VI.—TIME, 7 HOURS.

Contents of Tubes.	Pancreatic Juice. Minims.	Bile. Minims.	Undigested Casein. Grams.	Digested Casein. Grams.	Tube Numbers.
Milk, 20 c.c. Water, 20 c.c.	0	0	1.705	0	1
Milk, 20 c.c. Water, 20 c.c.	8	10	1.035	.670	2
Milk, 20 c.c. Water, 20 c.c. HCl Dilute, m. ½	8	10	.889	.816	3
Milk, 20 c.c. Water, 20 c.c. HCl Dilute, m. ½	8	10	1.005	.690	4
Milk, 20 c.c. Maltose Solution, 20 c.c.	8	10	.905	.790	5

EXPERIMENT VII.—TIME, 5 HOURS.

Contents of Tubes.	Pancreatic Juice. Minims.	Bile. Minims.	Undigested Casein. Grams.	Digested Casein. Grams.	Tube Numbers.
Milk, 15 c.c. Water, 15 c.c.	0	0	1.060	0	1
Milk, 15 c.c. Water, 15 c.c.	8	10	.640	.420	2
Milk, 15 c.c. Water, 15 c.c. HCl Dilute, m. ½	8	10	.579	.481	3
Milk, 15 c.c. Water, 15 c.c. HCl Dilute, m. ½.	8	10	.560	.500	4
Milk, 15 c.c. Lime Water, 15 c.c.	8	10	.609	.451	5
Milk, 15 c.c. Maltose Solution, 15 c.c.	8	10	.639	.421	6

EXPERIMENT VIII.—TIME, 5 HOURS.

Contents of Tubes.	Pancreatic Juice. Minims.	Bile. Minims.	Undigested Casein. Grams.	Digested Casein. Grams.	Tube Numbers.
Milk, 15 c.c. Water, 15 c.c.	0	0	1.076	0	1
Milk, 15 c.c. Water, 15 c.c.	8	10	.569	.507	2
Milk, 15 c.c. Water, 15 c.c. HCl Dilute, m. ½	8	10	.536	.540	3
Milk, 15 c.c. Water, 15 c.c. HCl Dilute, m. ½	8	10	.529	.547	4
Milk, 15 c.c. Lime Water, 15 c.c.	8	10	.503	.573	5
Milk, 15 c.c. Maltose Solution, 15 c.c.	8	10	.556	.520	6

In order to facilitate the study of the questions involved in the above experiments, I have by grouping the tubes bearing upon the same subject, made a number of tables which will now be considered under appropriate headings.

INFLUENCE OF MALTOSE ON THE PANCREATIC DIGESTION OF CASEIN.

TABLE I.

Contents of Tubes.	Pancreatic Juice. Minims.	Bile. Minims.	Casein Digested. Gram.
Milk, 15 c.c.+Water, 15 c.c	8	10	.420
Milk, 15 c.c.+Maltose Sol., 15 c.c	8	10	.421
Milk, 15 c.c.+Water, 15 c.c	8	10	.507
Milk, 15 c.c.+Maltose Sol., 15 c.c	8	10	.520
Milk, 15 c.c.+Water, 15 c.c	8	10	.670
Milk, 15 c.c.+Maltose Sol., 15 c.c	8	10	.790
Milk, 15 c.c.+Water, 15 c.c	5	10	.428
Milk, 15 c.c.+Maltose Sol., 15 c.c	5	10	.435
Milk, 15 c.c.+Water, 15 c.c	5	10	.428
Milk, 15 c.c.+Maltose Sol., 15 c.c	5	10	.486
Milk, 15 c.c.+Water, 15 c.c	8	10	.510
Milk, 15 c.c.+Milk Sugar Sol., 15 c.c	8	10	.529
Milk, 15 c.c.+Water, 15 c.c	5	10	.428
Milk, 15 c.c.+Milk Sugar Sol., 15 c.c	5	10	.474

By a study of this table it will be noted that the pancreatic digestion of casein was in every instance slightly facilitated by the presence of a maltose solution, and that in Experiments VI and VII of this series, a milk sugar solution seemed to exercise the same favorable influence. The inference, therefore, from this table is that rabbits' pancreatic juice in the presence of bile is somewhat assisted in casein proteolysis by the presence of a maltose or milk sugar solution.

In a previous paper[1] I demonstrated the physiological fact that acid proteids undergoing digestion will slightly increase the diastatic action of rabbits' pancreatic juice. It would seem, therefore, from these observations that the inference may be drawn that both the diastatic and proteolytic action of rabbits' pancreatic juice goes on more rapidly when the juice is acting upon a mixture of starches and albumens than when the juice is acting separately upon these food-stuffs.

It must, however, be remembered that there are some diffi-

1 *American Journal of Physiology*, Vol. ii, No. 5.

culties in the way of applying these principles in the solution of the much discussed question of the value of gruels in infant feeding.

Jacobi has long taught that in healthy children milk digestion goes on more satisfactorily when it is mixed with a decoction of one of the cereals; and most of the recent writers upon the subject of children's feeding have come to agree with Jacobi, believing, as they do, that under the influence of these decoctions the rennet and hydrochloric acid of the stomach precipitate the casein in more flocculent clots, thus enabling the ferments to come in more intimate contact with the casein to be digested.[1] Whatever may be the explanation, however, I think we may possibly infer from the above experiments that the favorable influence of these cereal decoctions on casein digestion is continued even after the milk leaves the stomach and comes under the influence of the various digestive enzymes of pancreatic juice in the intestinal canal.

INFLUENCE OF LIME WATER ON THE PANCREATIC DIGESTION OF CASEIN.

TABLE II.

Contents of Tubes.	Pancreatic Juice. Minims.	Bile. Minims.	Casein Digested. Grams.
Milk, 15 c.c.+Water, 15 c.c	6	0	.554
Milk, 15 c.c.+Lime Water, 15 c.c	6	0	.652
Milk, 15 c.c.+Water, 15 c.c	5	10	.428
Milk, 15 c.c.+Lime Water, 15 c.c	5	0	.405
Milk, 15 c.c.+Water, 15 c.c	5	10	.428
Milk, 15 c.c.+Lime Water, 15 c.c	5	10	.542
Milk, 15 c.c.+Water, 15 c.c	8	10	.510
Milk, 15 c.c.+Lime Water, 15 c.c	8	10	.575
Milk, 15 c.c.+Water, 15 c.c	8	10	.420
Milk, 15 c.c.+Lime Water, 15 c.c	8	10	.451
Milk, 15 c.c.+Water, 15 c.c	8	10	.507
Milk, 15 c.c.+Lime Water, 15 c.c	8	10	.573

A study of this table indicates that lime water slightly increases the proteolytic action of rabbits' pancreatic juice on casein. The important rôle which lime water has long played in the milk feeding of infants has given it, in certain conditions, an empirical value which cannot be doubted. It is perhaps true that the beneficial results which are obtained from the use of lime water, in

1 Chapin, *Archives of Pediatrics*, December, 1899.

the gastric digestion of milk, are in part due, as Dr. Chapin said in a paper before this Society last year, to the fact that the action of rennet is facilitated by the presence of the salts of lime. It also, however, has some value in neutralizing the acidity which has almost always developed in dairy milk before it has reached the dwelling houses in our large cities. And may it not also be possible that the beneficial influence of lime water on the pancreatic digestion of casein is exerted in somewhat the same way? That is to say, in the milk feeding of infants the lime water, by facilitating the flaky deposit of casein in the stomach, causes the casein to come into the presence of the pancreatic juice in a more suitable form for active proteolysis, and it may even be conceived that the lime salts themselves may reach the intestine, there to stimulate the pancreatic digestion of casein.

INFLUENCE OF SODIUM CARBONATE ON THE PANCREATIC DIGESTION OF CASEIN.

TABLE III.

Contents of Tubes.	Pancreatic Juice. Minims.	Bile. Minims.	Casein Digested. Grams.
Milk, 15 c.c.+Water, 15 c.c	6	0	.554
Milk, 15 c.c.+.4 per cent. Sod. Carb. Sol., 15 c.c	6	0	.642
Milk, 15 c.c.+Water, 15 c.	5	10	.428
Milk, 15 c.c.+.4 per cent. Sod. Carb. Sol., 15 c.c	5	0	.541
Milk, 15 c.c.+Water, 15 c.c	5	10	.428
Milk, 15 c.c.+.4 per cent. Sod. Carb. Sol., 15 c.c	5	10	.585
Milk, 15 c.c.+Water, 15 c.c	10	0	.690
Milk, 15 c.c.+.4 per cent. Sod. Carb. Sol., 15 c.c	10	0	.697
Milk, 15 c.c.+Water, 15 c.c	6	0	.554
Milk, 15 c.c.+.8 per cent. Sod. Carb. Sol., 15 c.c	6	0	.653

The study of this table shows that the presence of sodium carbonate greatly increases the proteolytic action of rabbits' pancreatic juice on casein. This physiological observation is of importance because of the fact that sodium carbonate is a normal constituent of the succus entericus. One may infer, therefore, that the alkaline intestinal juice will facilitate the action of trypsin on casein. One cannot, however, say that the value of sodium carbonate in the milk feeding of children depends either wholly

or partly upon this physiological fact, since it is quite impossible to see how sodium carbonate could pass through the acid contents of the stomach and reach the intestinal canal in a condition to facilitate the pancreatic digestion of casein. The good that comes from sodium carbonate in infant feeding is probably due to the fact that it neutralizes the fermentation acids which have been formed in the milk.

INFLUENCE OF COMBINED HYDROCHLORIC ACID ON THE PANCREATIC DIGESTION OF CASEIN.

TABLE IV.

Contents of Tubes.	Pancreatic Juice. Minims.	HCl Dilute. Minims.	Casein Digested. Grams.
Milk, 15 c.c.+Water, 15 c.c	10	0	.690
Milk, 15 c.c.+Water, 15 c.c	10	½	.680
Milk, 15 c.c.+Water, 15 c.c	10	0	.690
Milk, 15 c.c.+Water, 15 c.c	10	½	.597
Milk, 15 c.c.+Water, 15 c.c	10	0	.690
Milk, 15 c.c.+Water, 15 c.c	10	1	.527
Milk, 15 c.c.+Water, 15 c.c	10	0	.690
Milk, 15 c.c.+Water, 15 c.c	10	1	.519

The few experiments recorded in this table indicate that combined hydrochloric acid slightly retards the proteolytic action of trypsin on casein. The retarding influence, however, is not very great, with the amount of acid here used, considerable proteolysis being accomplished by the pancreatic juice when one minim of dilute hydrochloric acid was added to fifteen cubic centimetres of milk.

If one refers to Experiment I in connection with the study of the following table, it is evident that bile not only neutralizes the retarding influence of combined hydrochloric acid on the pancreatic digestion of casein, but that by its presence it enables the pancreatic juice to do more work on acid casein than it could do on neutral casein, or on neutral casein mixed with bile. That is to say, bile assists the pancreatic juice in the digestion of casein, but it renders even greater assistance when the casein is partly saturated with hydrochloric acid. When, therefore, rabbits' bile and rabbits' pancreatic juice are brought in contact with acid casein, conditions are provided which favor the proteolytic action of trypsin on casein.

INFLUENCE OF BILE AND COMBINED HYDROCHLORIC ACID ON THE PANCREATIC DIGESTION OF CASEIN.

TABLE V.

Contents of Tubes.	Pancreatic Juice. Minims.	Bile. Minims.	HCl Dilute. Minims.	Casein Digested. Grams.
Milk, 15 c.c.+Water, 15 c.c	6	12	0	.581
Milk, 15 c.c.+Water, 15 c.c	6	12	½	.594
Milk, 15 c.c.+Water, 15 c.c	6	12	0	.581
Milk, 15 o.c.+Water, 15 c.c	6	12	½	.590
Milk, 15 c.c.+Water, 15 c.c	5	10	0	.428
Milk, 15 c.c.+Water, 15 c.c	5	10	½	.527
Milk, 15 c.c.+Water, 15 c.c	5	10	0	.428
Milk, 15 c.c.+Water, 15 c.c	5	10	½	.519
Milk, 15 c.c.+Water, 15 c.c	6	8	0	.458
Milk, 15 c.c.+Water, 15 c.c	6	8	½	.508
Milk, 15 c.c.+Water, 15 c.c	6	8	0	.458
Milk, 15 c.c.+Water, 15 c.c	6	8	½	.544
Milk, 15 c.c.+Water, 15 c.c	8	10	0	.510
Milk, 15 c.c.+Water. 15 c.c	8	10	½	.570
Milk, 15 c.c.+Water, 15 c.c	8	10	0	.510
Milk, 15 c.c.+Water, 15 c.c	8	10	½	.598
Milk, 15 c.c.+Water, 15 c.c	8	10	0	.670
Milk, 15 c.c.+Water, 15 c.c	8	10	½	.690
Milk, 15 c.c.+Water, 15 c.c	8	10	0	.670
Milk, 15 c.c.+Water, 15 c.c	8	10	½	.816
Milk, 15 c.c.+Water, 15 c.c	8	10	0	.420
Milk, 15 c.c.+Water, 15 c.c	8	10	½	.481
Milk, 15 c.c.+Water, 15 c.c	8	10	0	.420
Milk, 15 c.c.+Water, 15 c.c	8	10	½	.500
Milk, 15 c.c.+Water, 15 c.c	8	10	0	.507
Milk, 15 c.c.+Water, 15 c.c	8	10	½	.540
Milk, 15 c.c.+Water, 15 c.c	8	10	0	.507
Milk, 15 c.c.+Water, 15 c.c	8	10	½	.547
Milk, 15 c.c.+Water, 15 c.c	6	12	0	.584
Milk, 15 c.c.+Water, 15 c.c	6	12	1	.567
Milk, 15 c.c.+Water, 15 c.c	5	10	0	.428
Milk, 15 c.c.+Water, 15 c.c	5	10	1	.465
Milk, 15 c.c.+Water, 15 c.c	6	8	0	.458
Milk, 15 c.c.+Water, 15 c.c	6	8	1	.460
Milk, 15 c.c.+Water, 15 c.c	6	8	0	.458
Milk, 15 c.c.+Water, 15 c.c	6	8	1	.410

This table shows that the addition of a small percentage of hydrochloric acid almost invariably increases the proteolytic action of pancreatic juice upon casein, when the juice is acting in the presence of bile. And when one remembers that in the carnivora the duodenal contents are always acid, and that even in the herbivora a certain amount of hydrochloric acid is combined with the proteids as they are discharged from the stomach into the duodenum, and that the intestinal contents lose their

acidity and become alkaline in their reaction, only after they have passed down some distance from the pylorus (in the carnivora a longer distance than in the herbivora), then one can see the force of the above physiological propositions in explaining the digestion of milk in the intestinal canal of all animals, including man. In the infant of the human species, for example, let us suppose that the milk, after being subjected in the stomach to the influence of rennet, hydrochloric acid and pepsin, is discharged, partially digested, through the pylorus, into the duodenum; the casein being either wholly or partly saturated with hydrochloric acid is brought at once under the influence of a mixture of bile and pancreatic juice, and these conditions, as we have demonstrated, being most favorable to the pancreatic digestion of casein, proteolysis will go on rapidly. As the casein passes down the intestinal canal, it presently finds itself in an alkaline medium, the combined hydrochloric acid being wholly neutralized by the sodium carbonate and other alkalies found in the intestinal juices. In this alkaline medium, as we demonstrated in Table III, the trypsin still finds itself under conditions most favorable to its action, and proteolysis thus continues under favorable influences throughout the intestinal canal.

That a small amount of combined hydrochloric acid will, in the presence of bile, actually assist the proteolytic action of pancreatic juice on casein, is a physiological fact which has some bearing on the feeding of sick infants.

Jacobi, in speaking of infant feeding, says: "In acute and debilitating diseases which furnish no, or little, hydrochloric acid in the gastric secretion, a small quantity of the latter, well diluted, must be provided for." This is but one of many expressions I find, noting the value of hydrochloric acid in the feeding of sick children. In recent years my own clinical experience has taught me that hydrochloric acid is one of the most valuable agents we have in the treatment of diseases marked by feeble digestion in infants. Hydrochloric acid is, I believe, of special value, as Jacobi says, in those cases where malnutrition is pronounced, and the hydrochloric acid of the gastric juice is for this reason deficient. I have found it of value, however, in almost all cases where there is deficient casein digestion, as manifested by curds in the stools. Casein dilution, as Rotch has so clearly demonstrated, is the rational treatment of this condition. Yet

if we are to look to the proper nutrition of the infant, there is a limit to the amount of dilution which may be resorted to. In these cases I have often obtained the greatest benefit from the use of a pepsin hydrochloric acid solution. In my hospital wards I have used this mixture with great satisfaction in infants suffering from casein indigestion, due wholly or partly to a general malnutrition. These cases, as a rule, respond quickly to the acid, the curds diminish or disappear from the stool, and the infant is able to take and digest more milk. I wish, however, especially to note that the good effects of hydrochloric acid are not limited to these cases of malnutrition, but that it is also of real value in almost all cases of casein indigestion, whatever may be the cause, and whether the infant is being fed on breast milk, or some dilution of cow's milk.

In the light of the above experiments we can see that the beneficial action of hydrochloric acid is not confined to the stomach, but as combined hydrochloric acid it is continued in the intestinal canal, where it not only aids the pancreatic digestion of casein, but also acts as an intestinal antiseptic. It is my belief that a small portion of hydrochloric acid combined with proteids will, under certain conditions, aid the action of the enzymes of pancreatic juice, while at the same time it exercises a restraining influence on fermentations carried on by organized ferments.

In closing this paper I wish to add a note on certain changes which take place in the cream of milk when subjected to the combined influence of bile and pancreatic juice.

In the experiments above recorded I noted at the close of certain experiments, that free fat, or butter, was found floating on the surface of all those digestive mixtures, in which the milk had been subjected to the action of both bile and pancreatic juice. In other words, it was noted that the physiological emulsion of fats, as it occurs in milk, was partially destroyed by the combined action of bile and pancreatic juice, but that this emulsion was not destroyed by the action of either one of these agents when acting alone. This observation suggests the possibility that the emulsion of fats in milk is wholly or partially destroyed by the action of bile and pancreatic juice in the intestinal canal prior to their absorption. If it be true that the milk emulsion is destroyed in the intestinal canal, and the fats set free, we can

readily understand how in certain diseases of the intestinal canal, which interfere with the absorption of foods, we may have, even in milk-fed infants, greasy or fatty stools.

I wish again in this paper, as I have in previous ones, to acknowledge the skillful assistance of Dr. F. A. Southgate. I am also indebted to Dr. Dudley Webb for valuable assistance.